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STUDIES ON THE SYNTHESIS OF ENDOPHOSPHONOPEPTIDES.

Submitted by

NIGEL VICKER

For the degree of Ph.D

of the University of Bath

1984

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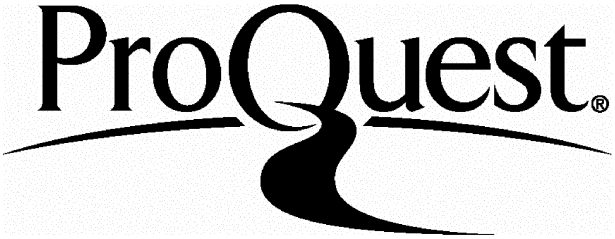

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TO MY PARENTS

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Finally I would like to thank Mrs. V. Edwards for her efficient and accurate typing of this thesis.

FOREWORD

The following abbreviations occur in the text.

Ac	Acetyl
ACE	Angiotensin Converting Enzyme
Ala	Alanine
Aq	Aqueous
B.p.	Boiling point
^{t}Bu	Tertiary-butyl
CPA	Carboxypeptidase A
DCC	N,N-dicyclohexylcarbodiimide
DCU	Dicyclohexylurea
DPPCl	Diphenylphosphinyl chloride
DMF	N,N-Dimethylformamide
DMSO	Dimethylsulphoxide
^{n}Dec	n-decyl
Et	Ethyl
Ft	Phthalimido
Gly	Glycine
Glu	Glutamic acid
GPI	Guinea pig ilium
hr	hour
^{n}Hex	n-hexyl
H.P.L.C.	High Pressure liquid chromatography
I.R.	Infra-red
Leu	Leucine
Me	Methyl
Met	Methionine
m/z	Mass to charge ratio
min	minute

mmol	millimole
m.p.	melting point
M.S.	Mass spectrum
MVD	Mouse vas deferens
Nle	nor-leucine
N.M.R.	nuclear magnetic resonance
Ph	Phenyl
Phe	Phenylalanine
^{i}Pr	iso-propyl
R.t.	Room temperature
RVD	Rat vas deferens
T.l.c.	Thin layer chromatography
TMS	Trimethylsilane
TMSBr	Trimethylsilylbromide
Trp	Tryptophan
Tyr	Tyrosine
u.v.	ultra-violet
z	benzyloxycarbonyl

Summary

The work described in this thesis was conducted between October 1981 and September 1984 and is concerned with the synthesis of endophosphonopeptides as possible transition state surrogates.

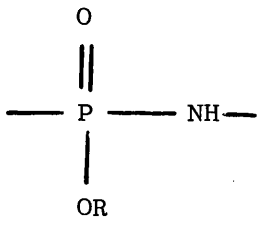
Various coupling methods for phosphonamide formation have been studied and several N-protecting functionalities evaluated in our synthetic strategy. The synthesis of an endophosphonoenkephalin an analogue of the naturally occurring pentapeptide leucine-enkephalin has been accomplished.

Contents

INTRODUCTION

I. Objectives

Structural variants of biologically important peptides are attracting increasing attention in the search of chemotherapeutically useful drugs. This research programme was concerned with the synthesis of peptide analogues in which key amide linkages were replaced by phosphonamide (1). The object was to synthesise endophosphonamide transition state surrogates for the cleavage of scissile peptide bonds.



(1) R = H or Alkyl

II. Synthesis of α-aminophosphonic and α-aminophosphinic acids

A range of synthetic routes to α-aminophosphonic acids has been developed, but novel strategies are necessary as emerging data points to their potential use as chemical regulators of biological mechanisms. The synthesis of α-aminophosphinic acids has been previously reviewed[1] and Redmore has published a comprehensive review of the chemistry of P-C-N systems.[2]

A. Mannich type procedures

Many such examples have been reported in the literature[3]. The reaction is that in which a phosphorus species has replaced the carbon nucleophile of the normal Mannich reaction. In 1952

Kabachnik and Medved[4] described the first synthesis of α-aminophosphonic acids from readily available starting materials, employing the reactions of aldehydes with ammonia and dialkylphosphites. Subsequently[5] they showed that a similar reaction occurred with ketones. The general reaction can be represented as:

$$R_2O_3PH + R^1R^2CO + NH_3 \longrightarrow R_2O_3P(O)(R^1)(R^2)NH_2$$

The methodology was further developed by improving yields and establishing conditions for hydrolysis of the phosphonate ester.[6] Fields[7] has investigated the mechanism of this reaction and showed that α-aminoalcohols yielded α-aminophosphonates on treatment with diethylphosphite. It was therefore suggested that α-aminoalcohols are likely intermediates in this process.

Phosphorous acid, formaldehyde and amines heated under strongly acidic conditions afforded good yields of α-amino-methylphosphonic acid.

The procedure, originally described by Moedritzer and Irani[8] may be represented by the equation:

$$R_{3-n}NH_n + nCH_2O + nHPO_3H_2 \longrightarrow R_{3-n}N(CH_2PO_3H_2)_n + nH_2O$$

However, the method is limited. Primary amines treated with one equivalent of formaldehyde and phosphorous acid yielded mixtures of mono- and di-phosphonic acid derivatives. With one exception, in the patent literature[9], formaldehyde has been the choice of

carbonyl component. Redmore[10] prepared several α-aminoaryl phosphonic acids by this procedure using arylaldehydes as the carbonyl components.

Polish workers[11] reported that phosphorous acid and benzylamine reacted with a range of carbonyl compounds, including acetone, propionaldehyde, and methyl ethyl ketone, affording the corresponding α-aminoalkylphosphonic acids. Reinvestigation of this work demonstrated[10] that the products exhibited different physical properties to authentic samples prepared by alternative routes.

Redmore[2] suggested a mechanism (Scheme 1) which was consistent with the experimental observations.

$$R_2NH + CH_2O \longrightarrow \underset{(2)}{R_2NCH_2OH} \longrightarrow \underset{(3)}{R_2\overset{+}{N}=CH_2} \longrightarrow \underset{(4)}{R_2NCH_2PO_3H_2}$$

(Scheme 1)

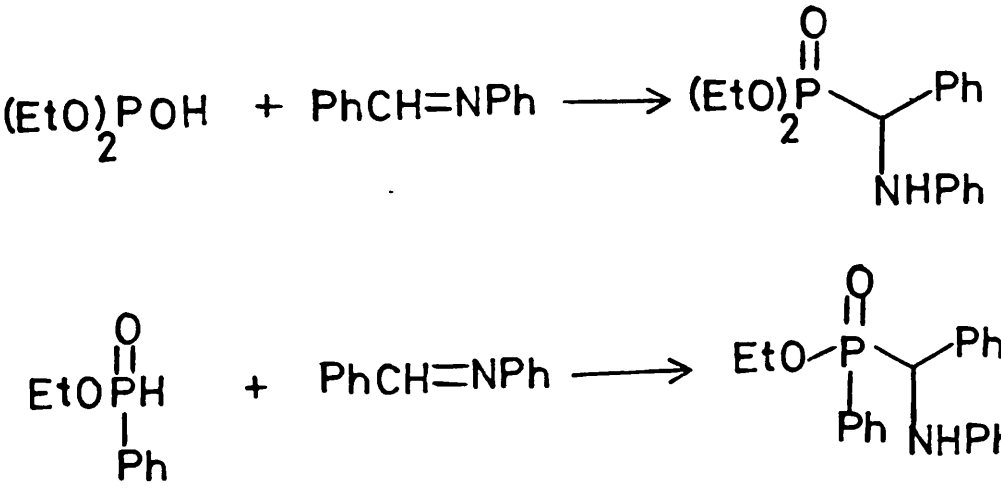
Thus, the amine reacted with formaldehyde to give the α-aminoalcohol (2) which subsequently eliminated water giving the iminium ion (3), and reaction with phosphorous acid yielded α-aminomethylphosphonic acid (4).

B. Addition of Phosphorus Nucleophiles to Carbon-Nitrogen Double Bonds.

Numerous examples of this procedure are described in the literature[3], and a range of substrates may be used. Aspects of the addition of phosphorus esters to unsaturated systems have been reviewed.[12] The following examples were selected to indicate the generality of this methodology.

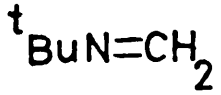
1. Imines

It has been shown[13,14] that dialkyl phosphites added to imines in the presence of a catalytic amount of sodium ethoxide. Phenylphosphinates may be prepared in an analogous fashion from alkylphenylphosphonites[15] (Scheme 2).



(Scheme 2).

The reaction of diethylphosphite and N-methylene-t-butyl amine (5) gave an α-aminophosphonate (6)[16], which was hydrolysed to the free α-aminophosphonic acid (7).



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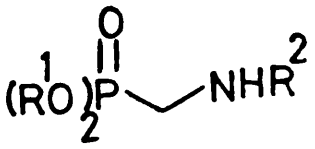
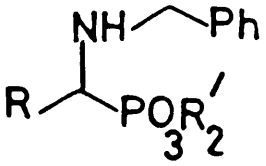


(6) R^1 = Et, R^2 = t_{Bu}

(7) R^1 = R^2 = H

Imine derivatives have been prepared with nitrogen protection that may be readily removed from the final product. Thus, the use of benzylamine[17] in the formation of the imine led to an N-benzyl- α-aminophosphonate. The free base was obtained upon catalytic hydrogenation (Scheme 3).

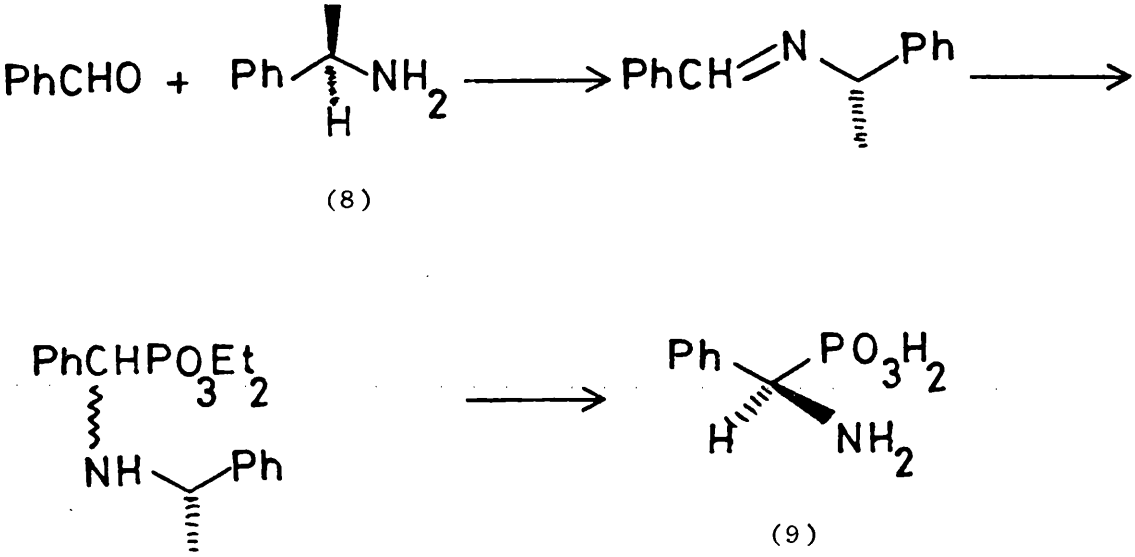


i, $(R^1O)_2POH$

(Scheme 3).

Derivatives in which alkyl substituents attached to the benzyl methylene gave products in which the amine functionality was more readily deprotected.[18,19,20] Diphenylmethylamine was used to prepare α-aminophosphonic[21] and α-aminophosphinic acids[22] containing a benzhydryl protected amino functionality which could be cleaved under mild conditions.

Both enantiomers of α-aminobenzylphosphonic acid (9)[23] were obtained using the dialkyl phosphite-imine approach. If either R(+)- or S(-)-2-methylbenzylamine (8) were employed an optically active α-aminobenzylphosphonic acid (9) was obtained (Scheme 4).

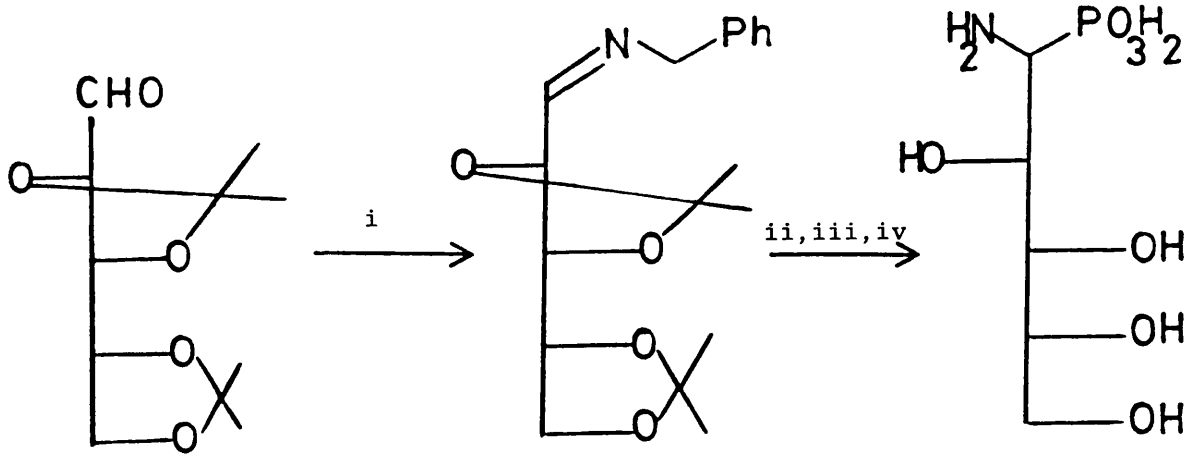


(8)

(9)

(Scheme 4)

Imines derived from carbohydrates reacted with dialkyl phosphites to give a tetrahydroxy-α-aminophosphonic acid[24] (Scheme 5).



i. $PhCH_2NH_2$; ii, $(RO)_2\overset{O}{\overset{\|}{P}}H$; iii, H_2/Pd; iv, HBr/HOAc.

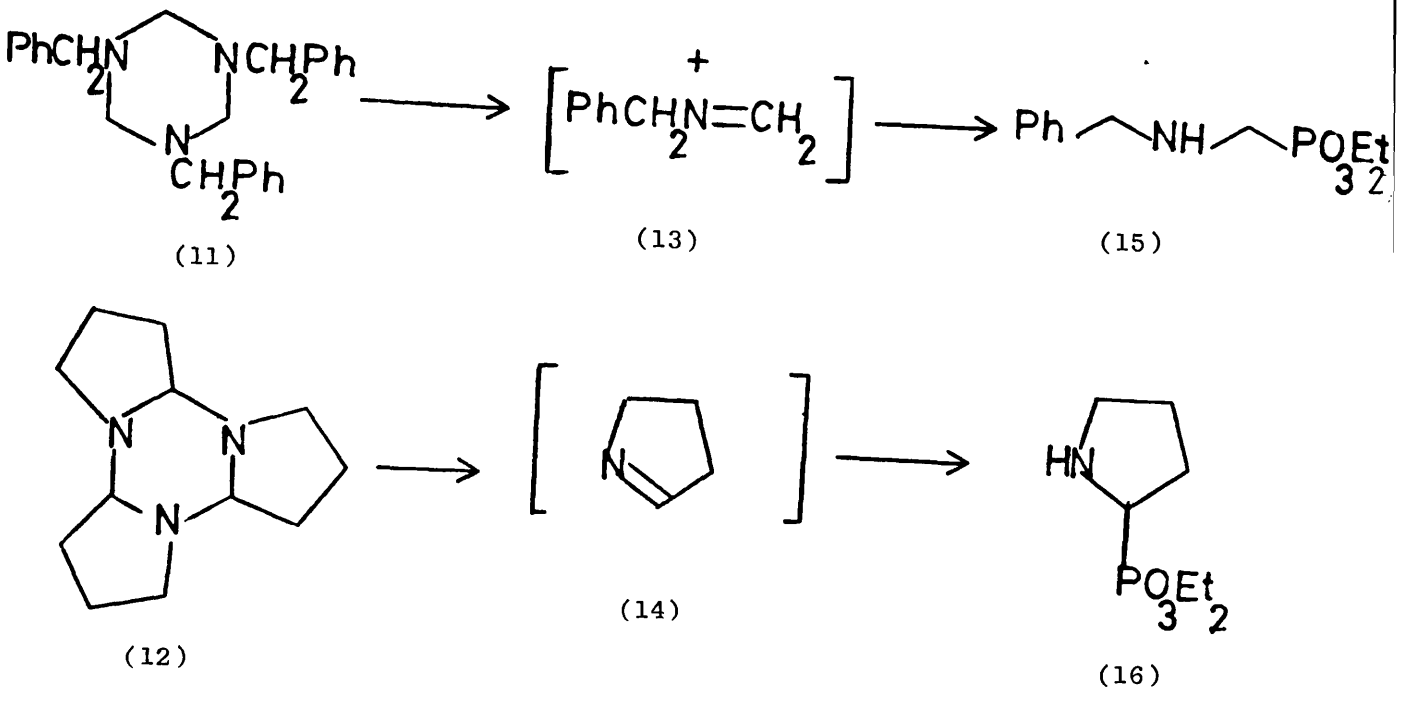
(Scheme 5).

Reactive N-fluorosulphonyl intermediates (10) and dialkyl phosphites reacted[25] giving good yields of the α-aminophosphonate and thence the corresponding α-aminophosphonic acid.

$$RCH{=}NSO_2F$$

(10)

The reactions of 1,3,5-tribenzylhexahydrotriazine[26] (11) and pyrroline trimer[27] (12) with dialkyl phosphites were postulated to occur via imine intermediates (13) and (14) respectively, (Scheme 6).

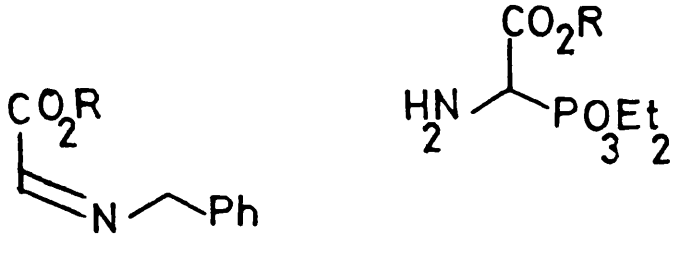


(11) (13) (15)

(12) (14) (16)

(Scheme 6)

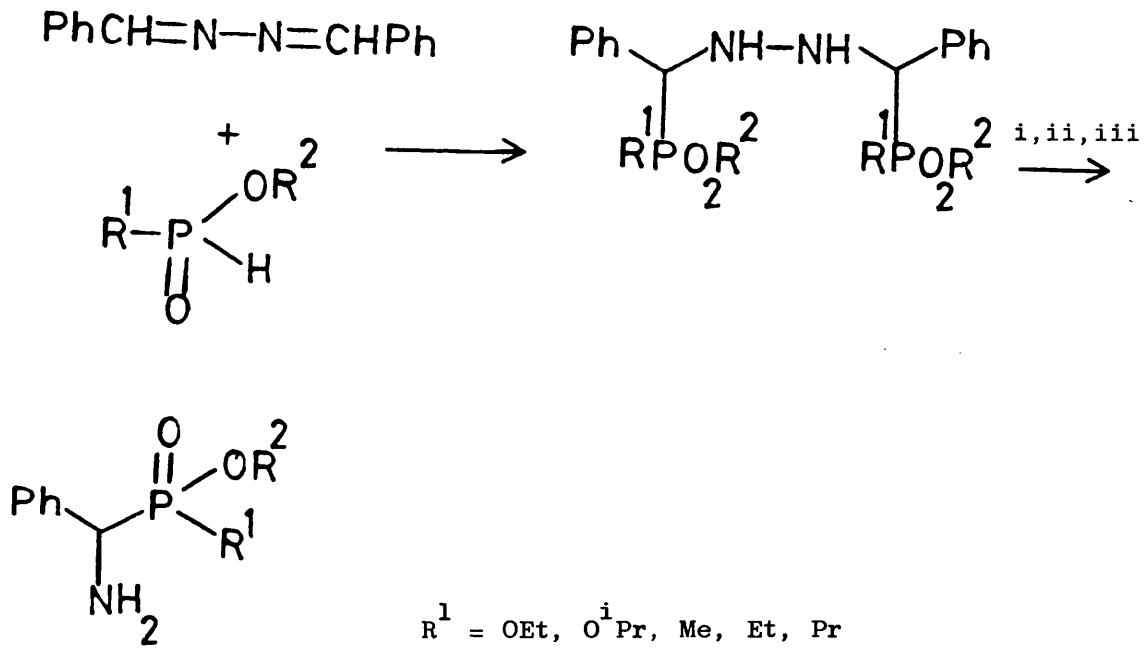
Thus (11) and (12) acted as precursors to imines (13) and (14) and reacted with diethyl phosphite giving α-aminophosphonates (15) and (16) respectively. Just[28] described an expedient route from (17) to (18), an important intermediate in the Merck[29] synthesis of cephalosporins.



(17) (18)

2. Aldazines and Ketazines

The reaction of aromatic aldazines with dialkyl phosphites[30,31,32] and alkyl phosphites[33] gave α-amino-phosphonates and α-aminophosphinates respectively (Scheme 7). Similarly aliphatic aldazines gave α-aminoalkylphosphonates[34,35] and α-aminoalkylphosphinates[36].



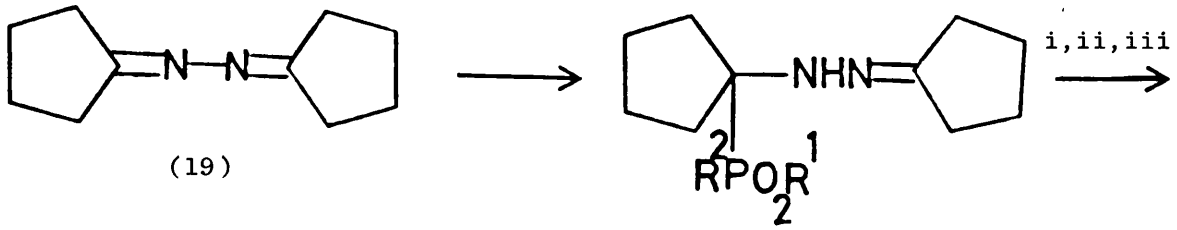
R^1 = OEt, O^iPr, Me, Et, Pr

R^2 = Et, ^iPr

i, Na cat; ii, HCl-AcOH; iii, propylene oxide

(Scheme 7)

A ketazine[37] was employed in an analogous reaction. Thus, cyclopentanone ketazine (19) led to α-aminophosphinic acid analogues (20) of cycloleucine (Scheme 8).



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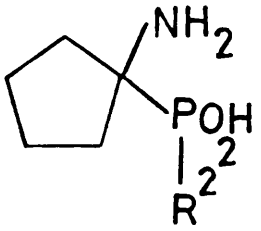


(20)

R^1 = Et, Me

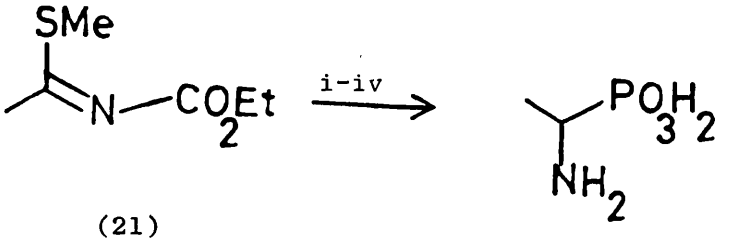
R^2 = OMe, Me

i, H_2-Ni; ii, HCl-AcOH; iii, propylene oxide

(Scheme 8)

3. Isothioacetamides

Nitriles were employed in a route to 1,1-dialkyldiphosphonic acids[38] but have not, until recently, been used to prepare α-aminophosphonic acids. N-Carboxy-S-methyl-iso-thioacetamide (21)[39] was prepared by heating acetonitrile with methanethiol under acidic conditions. Reacting the product with ethyl chloroformate to give (21) further elaboration of which yielded α-aminophosphonic acid (22) (Scheme 9). This procedure was used to synthesise both enantiomers of 1-amino-2-phenylethyl-phosphonic acid[40].



(21) (22)

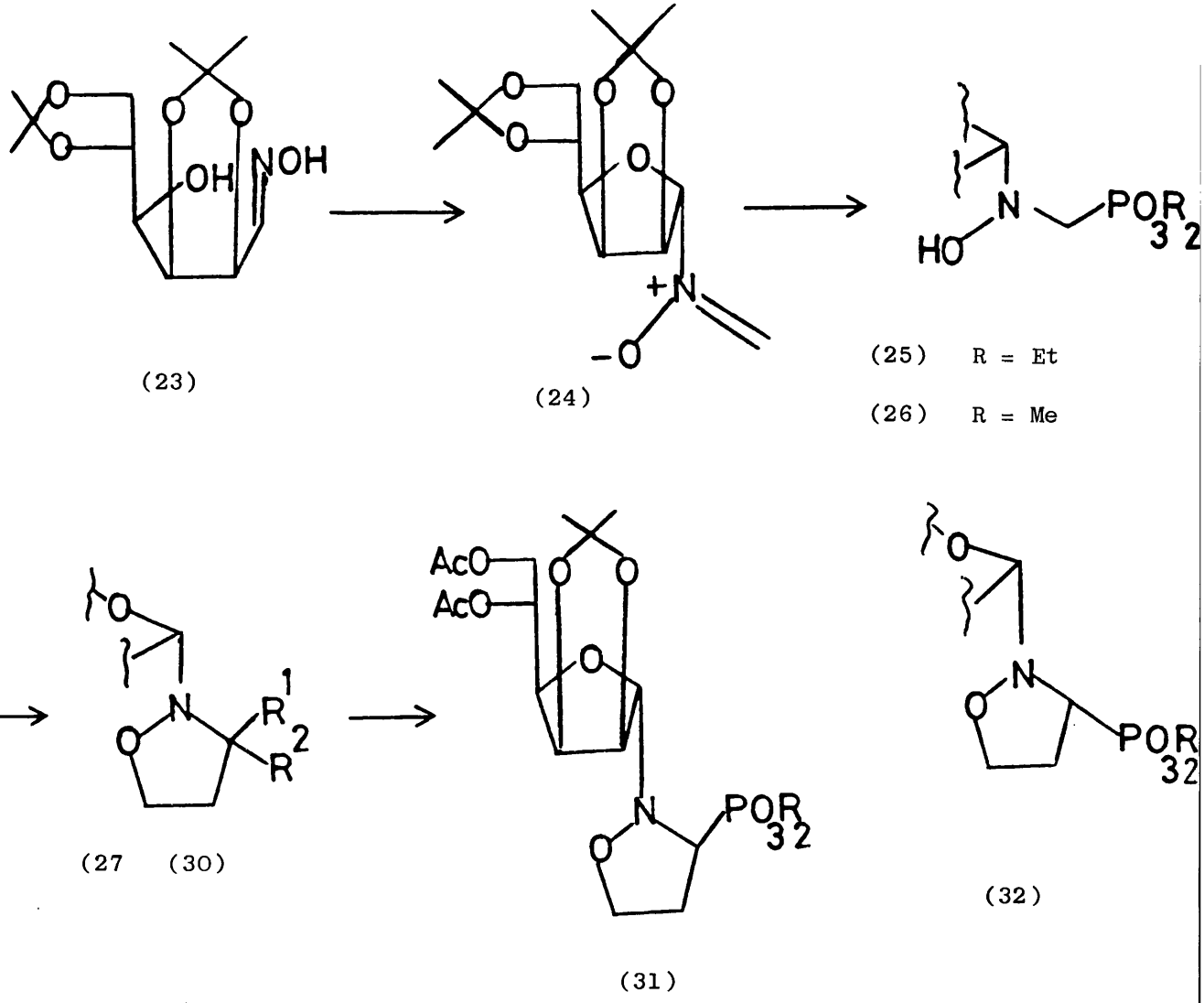
i, $NaPO_3Et_2$; ii, H_2-Ni; iii, HBr; iv, propylene oxide

(Scheme 9)

4. Nitrones

The addition of dialkyl phosphites to nitrones led to an asymmetric synthesis of four α-aminoalkylphosphonic acids[41] (Scheme 10).

Treatment of diethyl or dimethyl phosphite with nitrone (24), prepared in situ from the oxime (23) and formaldehyde, gave (N-glycosyl)hydroxyaminophosphonates (25) and (26) in excellent yields. Oxidation of (25) and (26) in the presence of ethene afforded the cycloadducts (27 - 30) in a 1:1 ratio. Stereochemical assignments of the cycloadducts were made by comparing the proton NMR spectra and specific rotations of the diacetates (31) and (32) with those of the corresponding α-amino acid derivatives of known configuration.



(23) (24) (25) R = Et (26) R = Me

(27 (30) (31) (32)

(27) $R^1 = PO_3Et_2$, $R^2 = H$

(28) $R^1 = H$, $R^2 = PO_3Et_2$

(29) $R^1 = PO_3Me_2$, $R^2 = H$

(30) $R^1 = H$, $R^2 = PO_3Me_2$

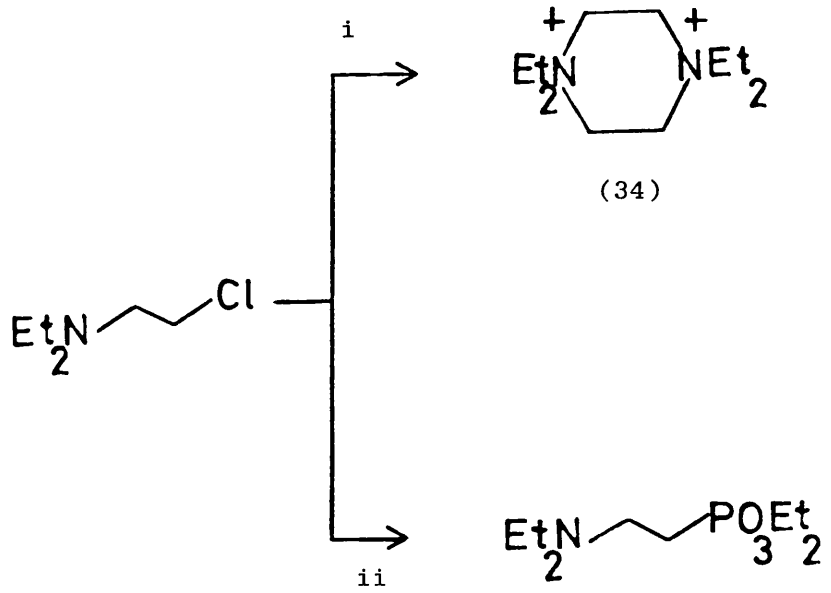
(Scheme 10).

The results indicated preferential formation of the L-isomers. Subsequently (30) and (32) were transformed into aminophosphonic acid analogues of L-5-oxaproline, L-homoserine, L-aspartic acid and L-asparagine.

C. Procedures which occur via Michaelis-Becker or Arbuzov Reactions

The Michaelis-Becker reaction in which a dialkyl phosphite, alkyl phosphonite or phosphonite anion is used to displace a halide from an alkyl halide complements the Arbuzov reaction.

An interesting example[42] is the preparation of N,N-diethyl-2-aminophosphonate (33) (Scheme 11). In this case the Arbuzov reaction yielded only the dimerised amine (34) whereas the Michaelis-Becker reaction afforded the desired aminophosphonate (33).



(34)

(33)

i, $P(OEt)_3$, ; ii, $NaPO_3Et_2$, RT.

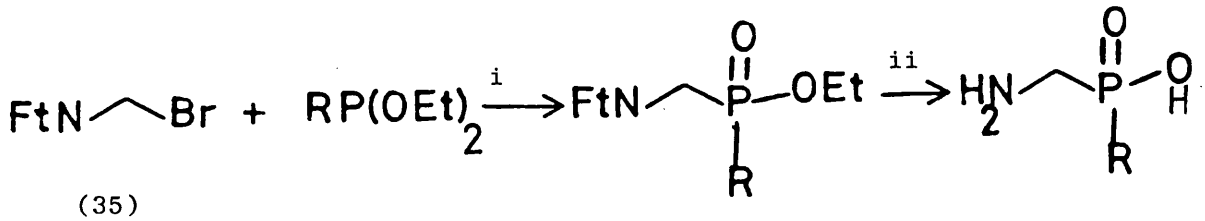
(Scheme 11).

Squibb researchers[43] prepared optically active 2-aminoalkylphosphonic acids by displacing an O-tosyl group with sodium dialkylphosphonates or sodium alkylphosphinates.

1. Arbuzov Reaction with Alkyl Halides

The Arbuzov reaction[44,45] in which a trivalent phosphorus ester reacts with an alkyl halide, is one of the principal synthetic methods for the formation of carbon phosphorus bonds. The process involves a valency expansion of phosphorus and the driving force for the reaction is the formation of the very strong phosphonyl P=O bond (627 kJ mol^{-1}).

Arbuzov reactions of N-bromomethylphthalimide (35) with phosphites or phosphonites gave α-aminomethylphosphonic (36) and α-aminomethylphosphinic (37)[46] respectively (Scheme 12).



(35)

(36) R = OH

(37) R = Me

i, heat; ii, HBr-AcOH

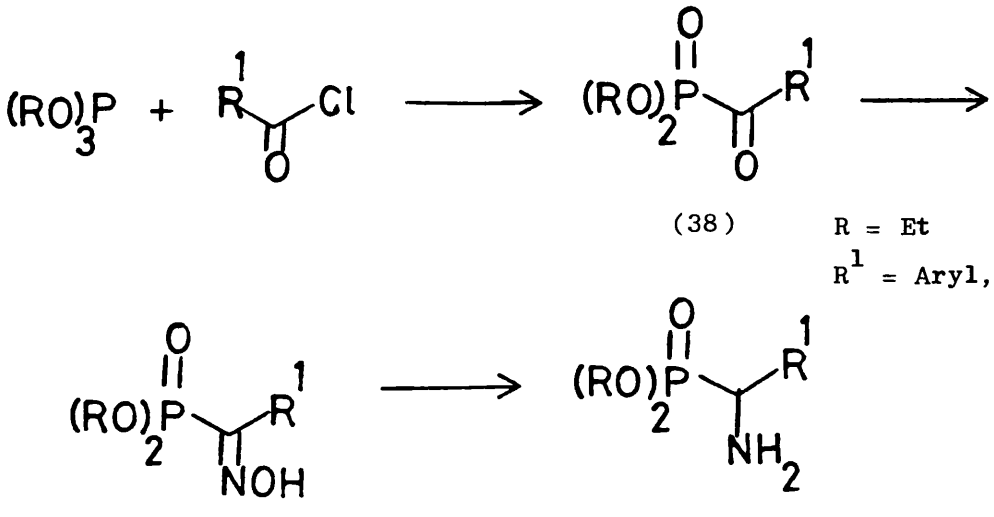
(Scheme 12).

The synthesis of α-aminophosphonates and α-aminophosphinates by this procedure is somewhat limited by the availability of the requisite halide.

2. Arbuzov Reactions with Acyl Halides

Trialkyl phosphites readily react with acyl halides producing acyl phosphonates[47]. In one of the first syntheses of α-aminophosphonates Kosolapoff[48] prepared diethylbenzoylphosphonate. After conversion to its p-nitrophenylhydrazone, it was reduced using aluminium-amalgam.

More recently it has been observed that dialkyl aroyl phosphonates and dialkyl acylphosphonates (38) may be converted to the corresponding oximes by treatment with hydroxylamine hydrochloride[49]. Reduction of the aryloximes with aluminium-amalgam afford α-aminoarylphosphonates (39, R^1 = aryl) and reduction of the alkyl oximes using Raney nickel gave α-aminoalkylphosphonates (39, R^1 = alkyl). (Scheme 13).



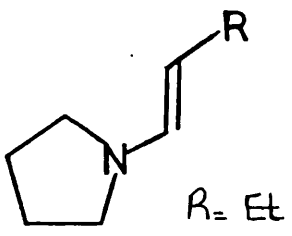
(Scheme 13).

A procedure was devised in which acylphosphonates were converted to their O-methyl oximes[50], reduction of which to α-aminoalkylphosphonates was achieved with diborane. This approach was employed[51] in a synthetic route to several α-aminophosphonic acids, including some analogues of naturally occurring amino acids. Acylphosphonates have also been converted[52] to α-aminoalkylphosphonic acids *via* the corresponding hydrazones.

3. Arbuzov Reaction and Michaelis-Becker Reactions of α,β-Unsaturated compounds

Chambers and Isbell[53] used acetamidoacrylic acid (40) in an Arbuzov reaction to obtain the aspartic acid analogue (41). Subsequently, the procedure was improved[54] and the use of diethyl acetamidomethylenemalonate (42), giving a further analogue of aspartic acid (43) was described. (Scheme 14).

Pyrrolidine enamine (44) reacted with diethyl phosphite to give α-aminophosphonate (45). It was noted that the corresponding enamine hydrochloride reacts with sodium diethyl phosphite to give (45) in greater yields[55].



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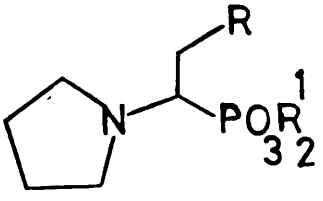

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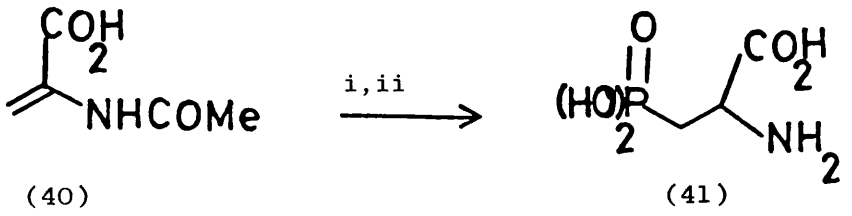

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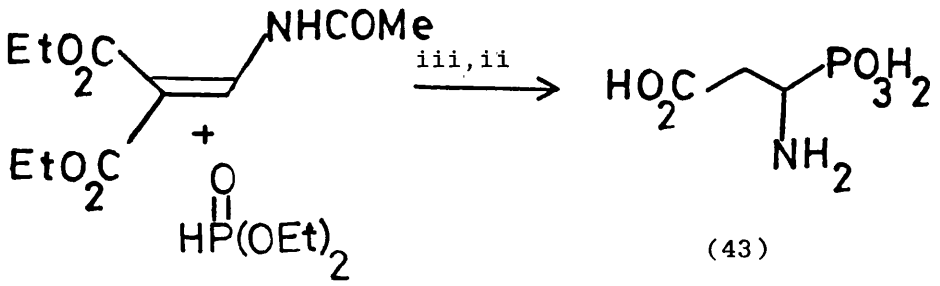


(42) (43)

i, $P(OEt)_3$, 120^{o};

ii, $HCl-H_2O$, 100^{o}; iii, NaOEt

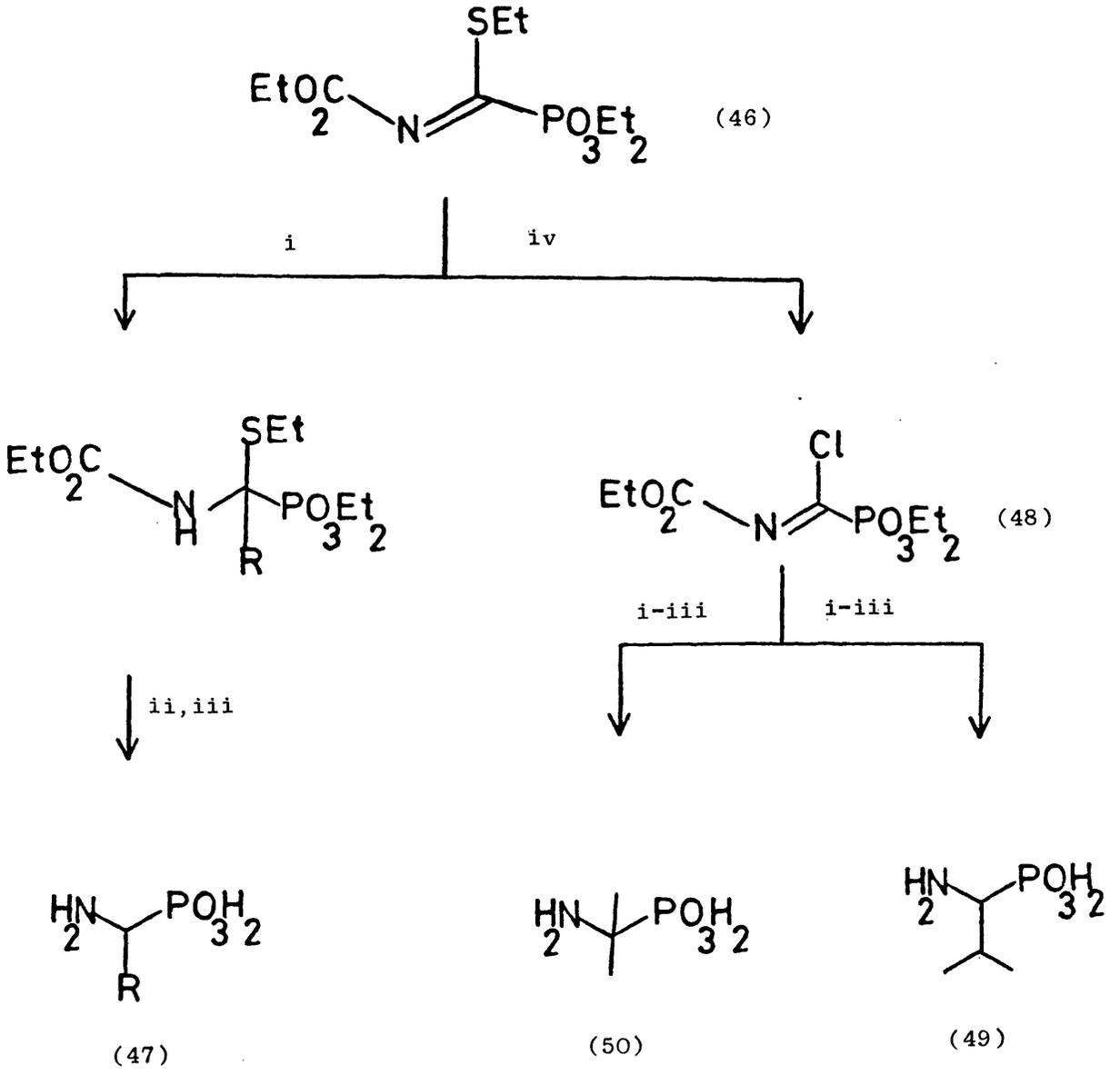
(Scheme 14).

4. Isothiocyanates in Arbuzov Reactions

The preparation[56] of N-ethoxycarbonylisothiocyanate has been described. Subsequent addition of trimethyl phosphite gave O,O-dimethyl-1-(N-ethoxycarbonylimino)-1-thiomethyl methylphosphonate.

In a similar reaction[57] with triethyl phosphite, phosphonate (46) was obtained. Grignard reactions gave, after reductive desulphurisation and hydrolysis, alkylmethylaminophosphonic acids, (47).

In contrast, imido chloride (48), obtained by treating (46) with thionyl chloride, reacted with Grignard reagents to give mono- and dialkyl addition products (49) and (50) respectively, depending on the nature of the Grignard reagent (Scheme 15).

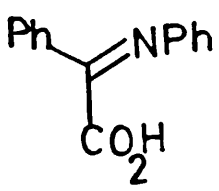


i, RMgX; ii, $NaBH_4$; iii, HBr; iv, $SOCl_2$

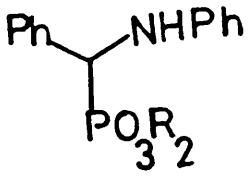
(Scheme 15).

5. Other Arbuzov Reactions

Reaction of trimethyl or triethyl phosphite with (N-phenylbenzimidoyl)-formic acid (51) generated the corresponding α-aminobenzylphosphonic acid (52)[58].

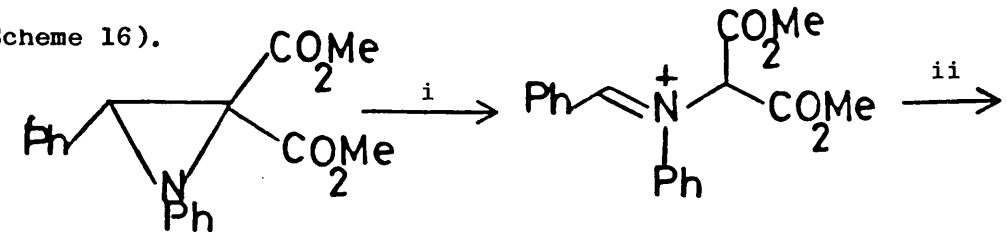


(51)

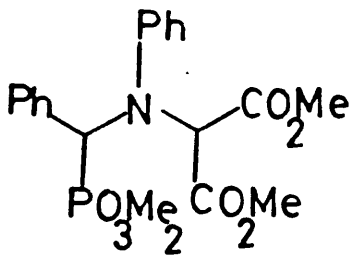


(52)

N-Substituted aziridines react with trimethyl phosphite[59] under acidic conditions to give α-aminophosphonates. The authors suggested that treatment of the aziridine with acid afforded an equilibrium mixture of intermediates and that the trimethyl phosphite reacted with an iminium species (53) producing α-aminophosphonate (54) via an Arbuzov type reaction (Scheme 16).



(53)

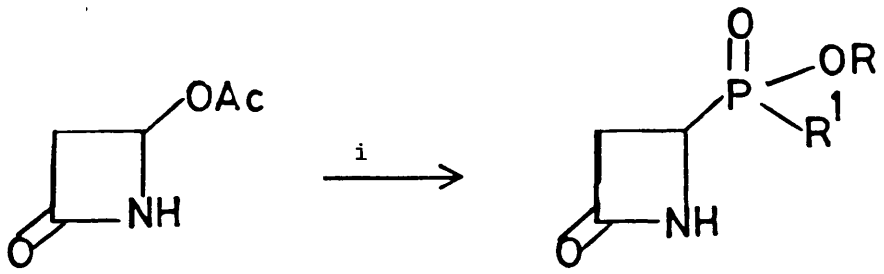


(54)

i, $PhCO_2H$; ii, $P(OMe)_3$

(Scheme 16).

Campbell and Carruthers[60,61] explored a quasi-Arbuzov reaction in acetate displacement from 4-acetoxyazetidin-2-one, obtaining a range of 4-oxoazetidin-2-ylphosphonates and phosphinates (55) (Scheme 17).



(55)

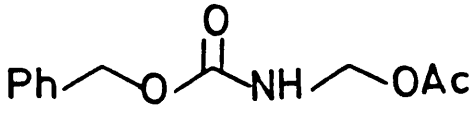
R	Me	Et	CH_2Ph	Et	CH_2Ph	CH_2CCl_3	Et
R^1	OMe	OEt	OCH_2Ph	Me	Me	Me	C CPh

i, $P(OMe)_3$

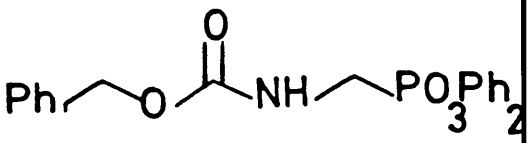
(Scheme 17).

These internally-protected α-aminoacids were then converted into phosphonoaspartic acids.

Displacement of acetate from (56) in a quasi-Arbuzov process, using triphenyl phosphite led to (57) and thence to diphenylphosphonoglycine[62].

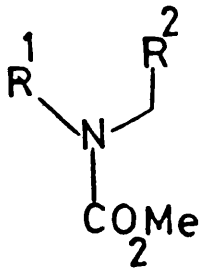


(56)

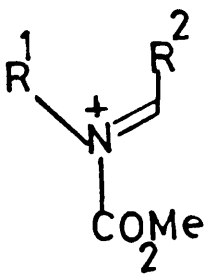


(57)

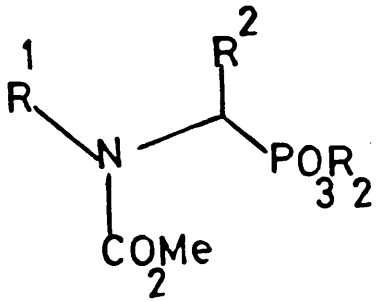
A new strategy involved formation of an acyliminium ion (59) from (58) by electrochemical means[63]. Trapping of (59) by phosphites yielded substituted aminophosphonic acids (60).



(58)



(59)



(60)

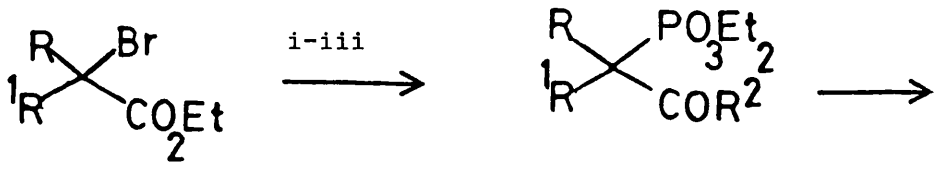
R^1, R^2 = H, alkyl, cycloalkyl.

D. Rearrangement Reactions

1. Curtius Reaction

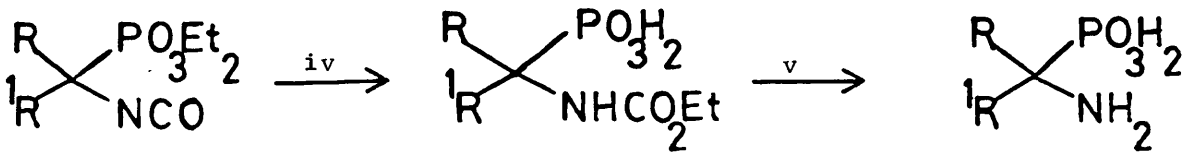
The Curtius rearrangement has been employed by Isbell[64] for the synthesis of α-aminophosphonic acids. Thus ethyl bromoalkanoates, (61) with triethyl phosphite, gave the diethylphosphonate (62) in an Arbuzov reaction. Treatment of phosphonates (62) with excess hydrazine afforded hydrazides (63) which were converted to the isocyanates (65) via acyl azides (64).

Hydrolysis of the isocyanates (65) either directly or via carbamates (66) gave α-aminophosphonic acids (67). (Scheme 18).



(61)

(62) R^2 = OEt
(63) R^3 = $NHNH_2$
(64) R^2 = N_3



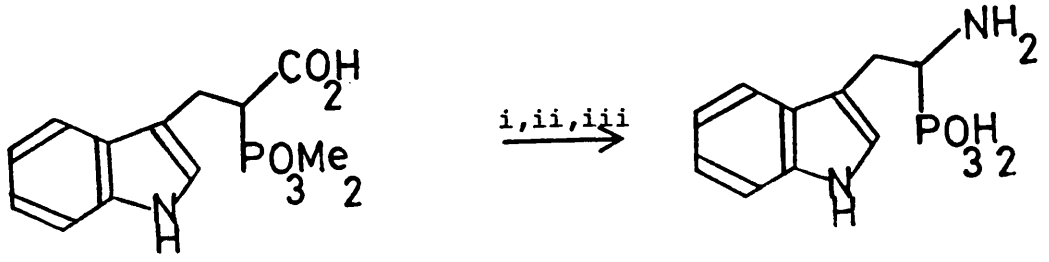
(65) (66) (67)

i, $P(OEt)_3$; ii, H_2NNH_2; iii, HCl, $NaNO_2$; iv, EtOH; v, HCl.

R	Me	Et	CH_2Ph	^nPr	^iPr	^nBu	^nHex	^nDec	Ph	Me
R^1	H	H	H	H	H	H	H	H	H	Me

(Scheme 18).

A recent application of the Curtius rearrangement may be found in Tischler's approach to D,L-phosphonotryptophan (68)[65] (Scheme 19).



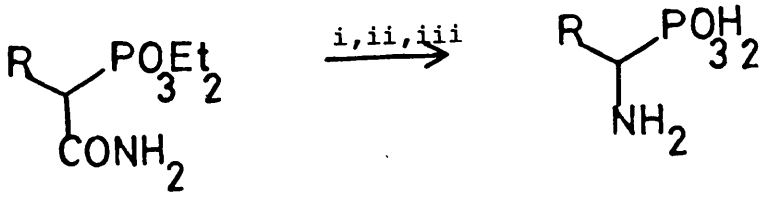
(68)

i, $(PhO)_2PON_3$; ii, $PhCH_2OH$; iii, HCl

(Scheme 19)

2. Hofmann rearrangement

Two groups have applied the Hofmann rearrangement to the synthesis of α-aminophosphonic acids. Whilst Rachon[66] only isolated α-aminophosphonic acids as products (Scheme 20).

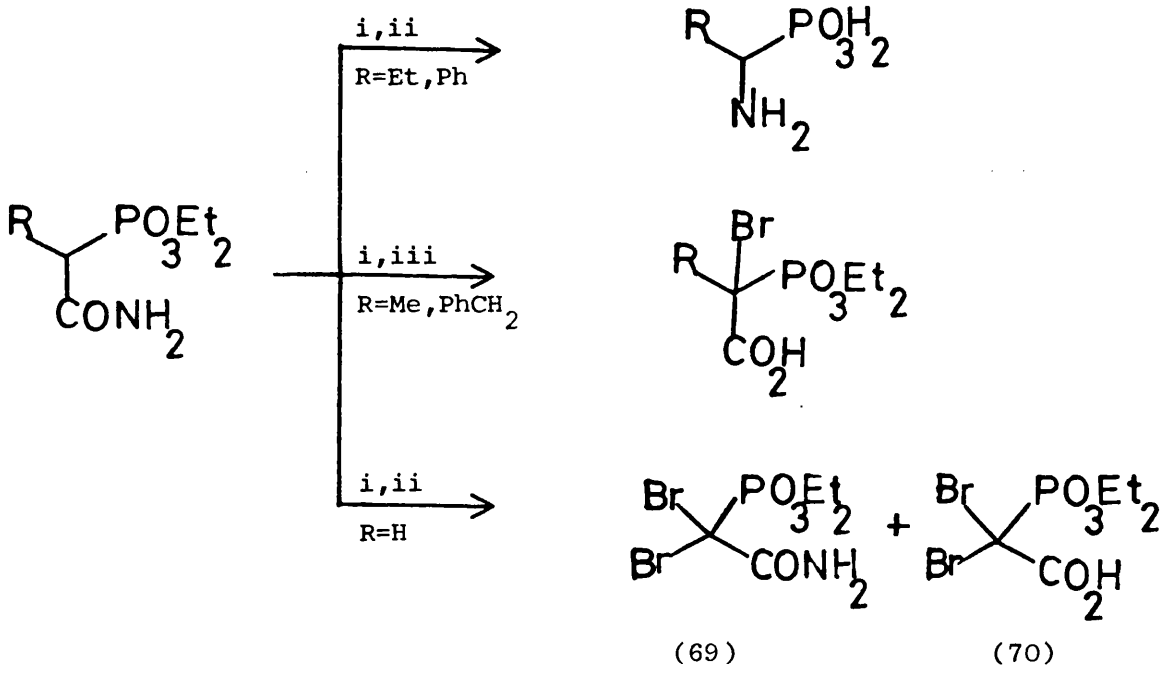


R = Me, Et, ^nPr, Ph

i, KOH, Br_2; ii, HCl-AcOH; iii, propylene oxide.

(Scheme 20).

Saroka and Mastalerz[67] found that the course of the reaction was dependent on the structure of the phosphonoacetamide starting material. Thus in only two instances was a Hofmann rearrangement observed. In other examples α-brominated products were formed. The parent phosphonoacetamide gave a mixture of dibromoacetamide (69) and dibromoacetic acid (70) (Scheme 21).



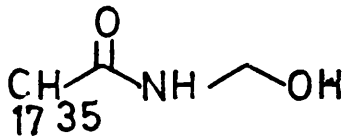
i, NaOBr-2N NaOH; ii, HCl; iii, 2N NaOH

(Scheme 21).

E. Other Methods of Preparing α-Aminophosphonic and α-Aminophosphinic Acids

1. Phosphorus trichloride

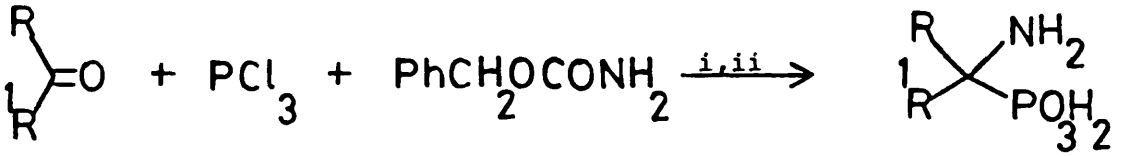
One of the earliest preparative methods for α-aminophosphonic acids utilised phosphorus trichloride[68]. Treatment of the α-hydroxyamide (71) with phosphorus trichloride and acetic acid, followed by hydrolysis, gave α-aminophosphonic acid (72).



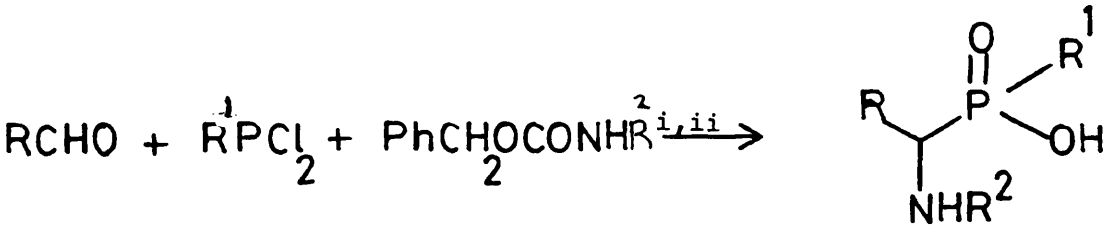
(71)

(72)

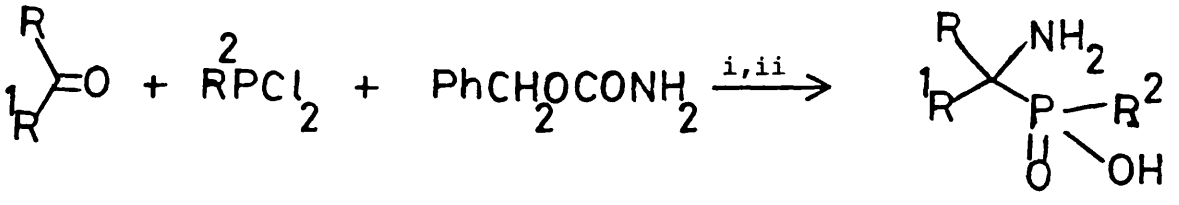
This method required long reaction times (12 hours to 7 days) and, consequently, phosphorus trichloride has not generally been exploited in the synthesis of α-aminophosphonic acids. One-pot procedures for the preparation of α-aminophosphonic, α-aminophosphinic acids[69] N-alkyl-α-aminophosphonic and N-alkyl-α-aminophosphinic acids[70] have now been described (Scheme 22).



R	Me	Ph	Me
R^1	H	H	Me



R	Me	Et	Me	^{i}Pr	Ph	Me	Ph
R^1	H	H	H	H	H	H	H
R^2	Ph	Ph	Et	Et	Et	Me	Me

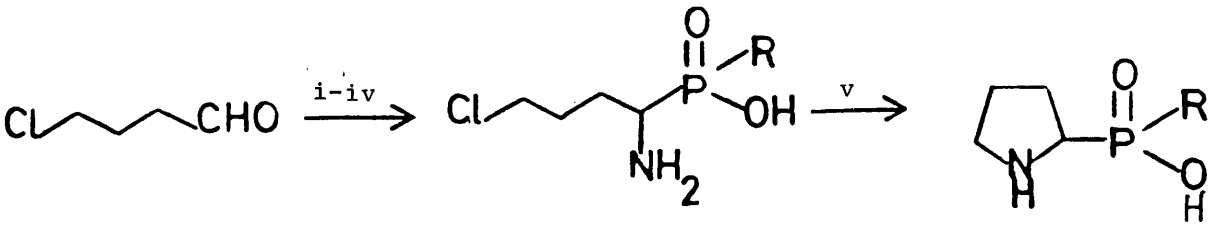


R	Me	Me	Me	^{n}Pr	Me	Me	Me	Me	^{i}Pr
R^1	Cl	Cl	Cl	Cl	Cl	Ph	Ph	Ph	Ph
R^2	Me	^{n}Pr	^{i}Pr	$PhCH_2$	$^{c}C_6H_{11}$	Me	^{n}Pr	^{i}Pr	Me

i, AcOH; ii, H_3O^+

(Scheme 22).

To date this method is one of the simplest for the preparation of free or N-alkyl substituted α-aminophosphonic or α-aminophosphinic acids. A notable application of this procedure is to be found in the synthesis of phosphonic and phosphinic analogues of proline (73)[70] (Scheme 23).



(73)

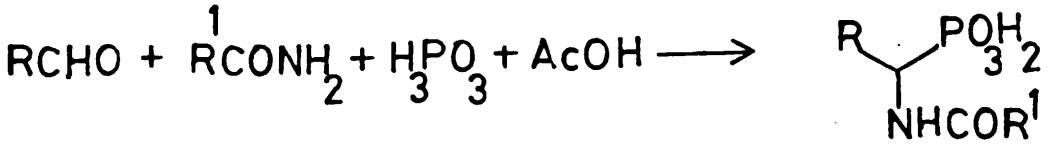
i, $PhCH_2OCONH_2$; ii, $RPCl_2$; iii, AcOH; iv, $HCl-H_2O$; v, NaOH

(Scheme 23).

However, this reaction is limited to aldehydes and carbamates derived from primary amines. Efforts to extend the methodology to ketones and benzyl-N,N-dialkylcarbamates have been unsuccessful[71].

2. Phosphorous Acid

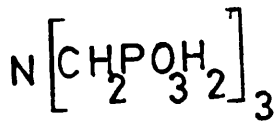
Apart from its use in Mannich type reactions phosphorous acid has also been utilised in other routes to afford α-aminoalkylphosphonic acids. Amidoalkylation of phosphorous acid yielded α-aminoalkyl and α-aminoarylphosphonic acids[72]. Phosphorous acid, amides and aldehydes reacted in acetic anhydride giving N-acylated α-aminophosphonic acids (Scheme 24).



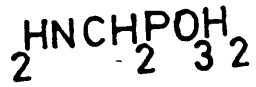
R	Me	Me	iPr	C_6H_5	$3\text{-}NO_2C_6H_4$	$4ClC_6H_4$	$4\text{-}MeC_6H_4$
R^1	$PhCH_2O$	Ph	$PhCH_2O$	Me	Ph	Me	Ph

(Scheme 24).

Patents described the conversion of a carboxylic acid into its phosphonic acid analogue using mixtures of phosphorous acid and phosphorus trichloride[73, 74]. Also mentioned was the conversion of nitrilotriacetic acid to its phosphonic acid analogue (74)[75]. More recently, the method was extended to N,N-dibenzylglycine which gave aminomethylphosphonic acid (72)[76].



(74)

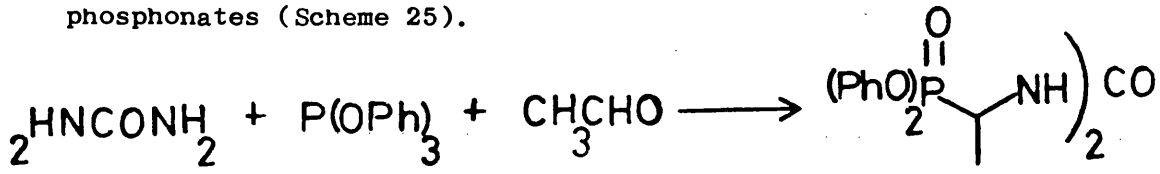


(72)

The preparation of aminomethylphosphonic acid by treating a mixture of phosphorous acid and trioxane in acetonitrile with phosphorus trichloride has been described in the patent literature[77].

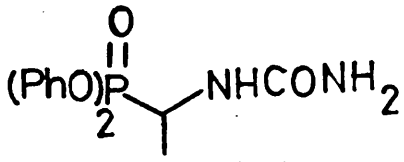
3. Use of Urea and Thiourea Derivatives.

The discovery by Birum[78] that urea and many mono- and di-substituted ureas and thioureas reacted with aldehydes and trivalent phosphorus esters giving α-ureidophosphonates, led to the development of a novel route to α-aminophosphonic acids. The reaction of urea with triphenylphosphite produces both diphosphonate (75) and monophosphonate (76). However, mono- and di-substituted ureas (77) and (78) gave only monophosphonates (79) and (80) respectively. Phosphonite esters were also found to react in a similar manner giving ureidophosphonates (Scheme 25).

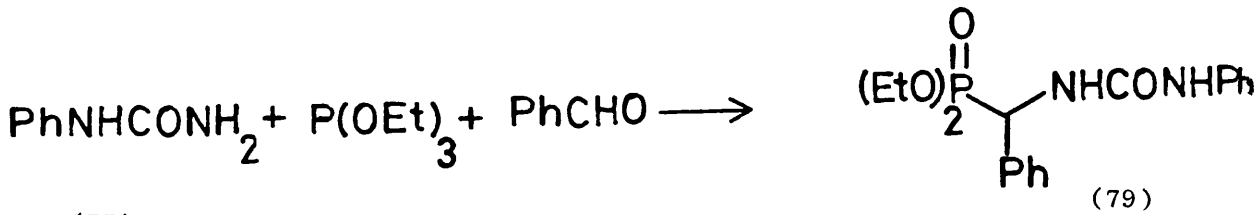


(75)

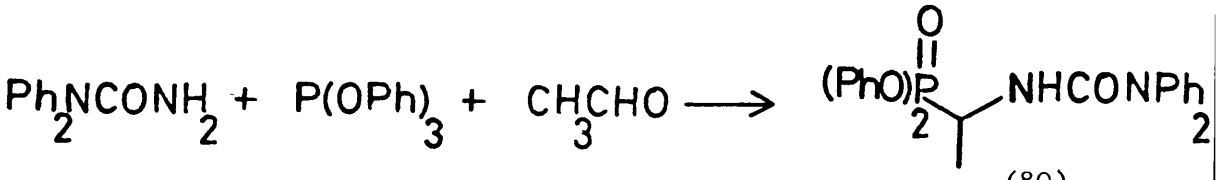
+



(76)



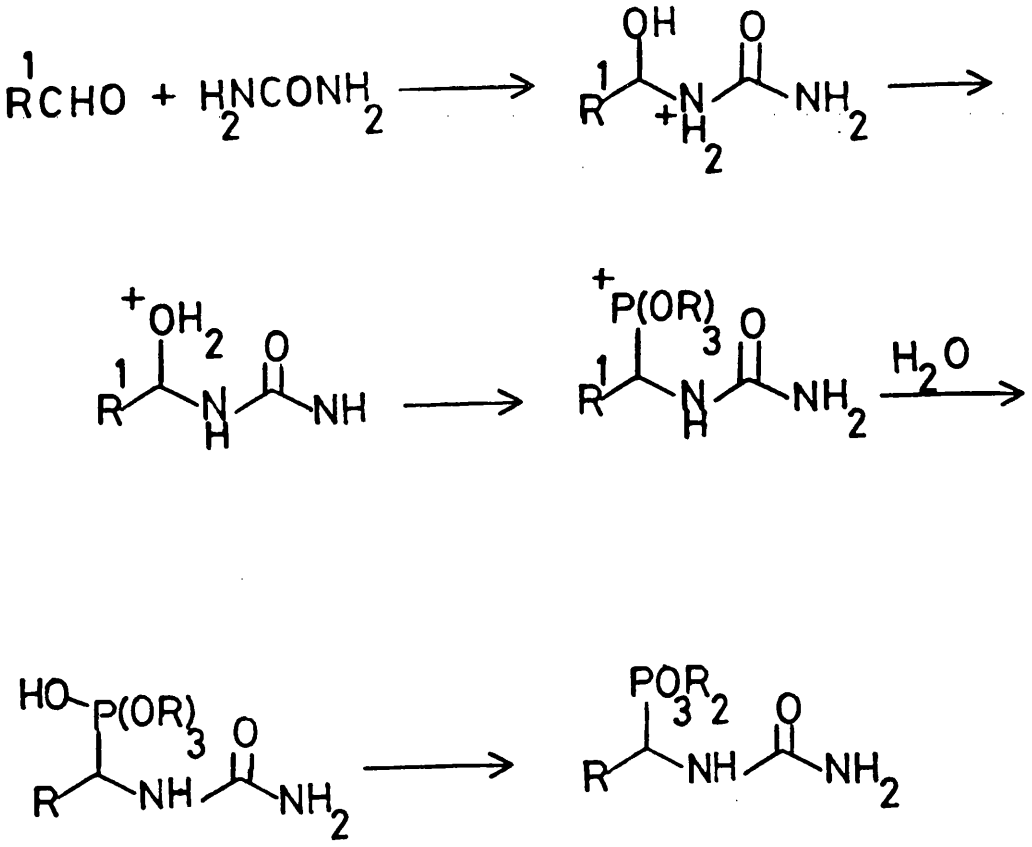
(77) (79)



(78) (80)

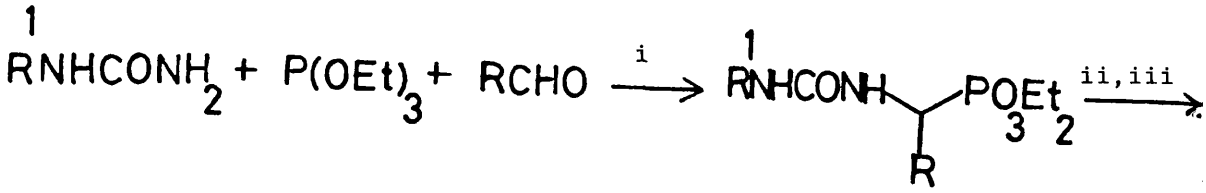
(Scheme 25).

An interesting observation was that triaryl phosphites were more reactive than trialkyl phosphites as trialkyl phosphites required acid catalysis before reaction occurred. A reversal in the order of reactivity was explained by the mechanism shown in Scheme 26. The first step in the formation of ureidophosphonates was the acid-catalysed reaction of urea and the aldehyde. Trialkyl phosphites are basic enough to inhibit the first step whereas the less basic triphenyl phosphite does not lower the acidity below the level needed for the reaction of the urea with the aldehyde.

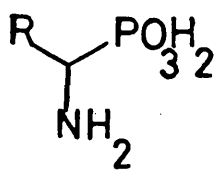


(Scheme 26).

Several applications of this procedure in the synthesis of α-aminophosphonic acids have appeared. Birum's reaction sequence was modified by hydrolysis of a ureidophosphonate (81)[79] (Scheme 27). More recently the reaction sequence was used to prepare both enantiomers of α-aminobenzylphosphonic acid[80].



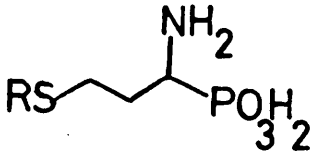
(81)



i, BF_3-Et_2O; ii, HCl-AcOH; iii propylene oxide.

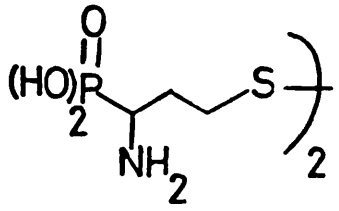
(Scheme 27).

Kudzin and Stec have elaborated earlier work with thioureas providing a "one-pot" synthesis of α-aminoalkylphosphonic acids *via* the thioureidoalkane phosphonates[81]. The method was applied to the syntheses of phosphonic acid analogues of homocysteine (82)[82], homocystine (83)[82] cysteine (84)[83] and cystine (85)[83].

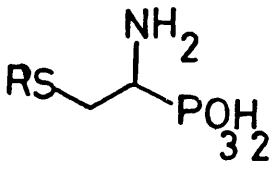


R=alkyl, CH_3CO, $(^tBuO)_3Si$

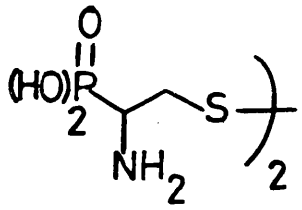
(82)



(83)



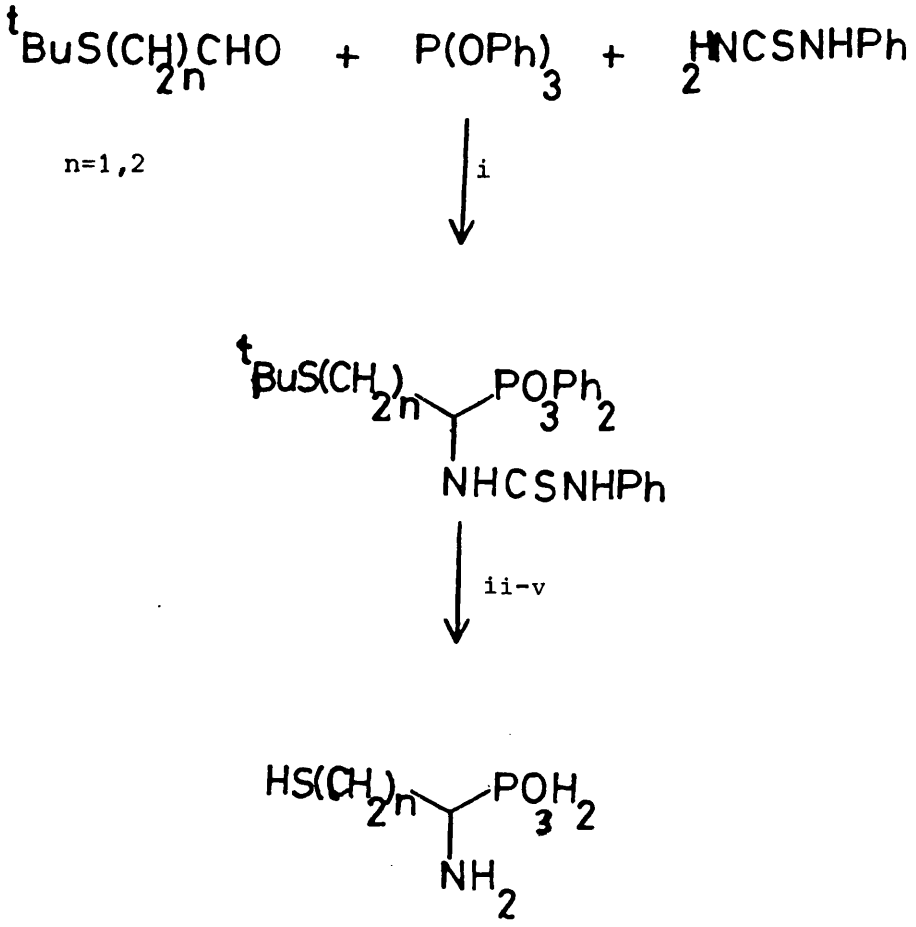
(84)



(85)

R = alkyl, benzyl

More recently, isolation of the free thiols (86) and (87) via their mercury salts has been described[84], (Scheme 28). An identical sequence was employed[85] to prepare phosphonic acid analogues of homocystine, methionine and ethionine.



(86) n=1
(87) n=2

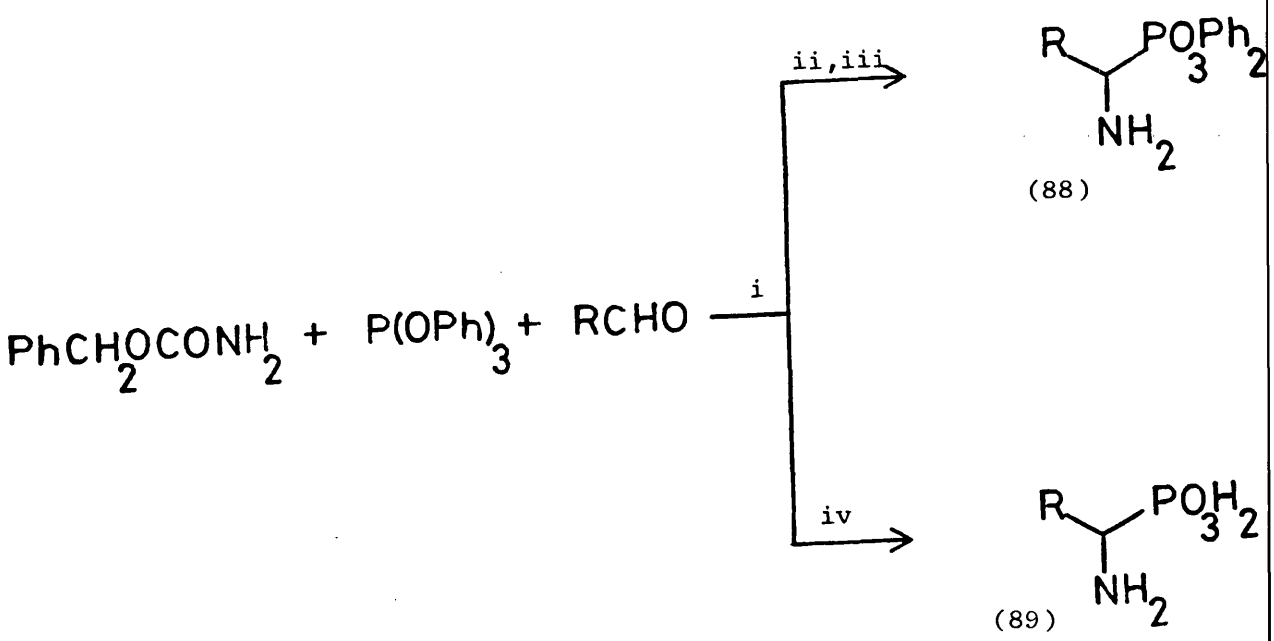
i, AcOH; ii, AcOH-HBr; iii, $HgSO_4$-H_2SO_4; iv, H_2S;

v, ion exchange.

(Scheme 28).

4. Carbamates

Reaction of triphenyl phosphite with benzyl carbamate gave either diphenylaminoalkylphosphonates (88)[86] or the corresponding free phosphonic acids (89)[87] depending on the method of hydrolysis (Scheme 29).

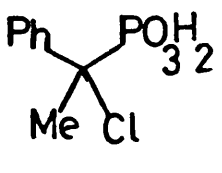


i, AcOH; ii, HBr-AcOH; iii, NH_3-Et_2O; iv, HCl

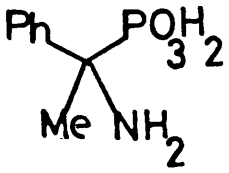
(Scheme 29).

5. Halomethyl Phosphorus Species

Nucleophilic displacement reactions on halomethylphosphorus species have received little attention, probably because of the difficulties associated with the desired displacement. Kosolapoff[88] prepared 1-methyl-1-phenyl-1-aminophosphonic acid (91) in low yield from the corresponding chloro-compound (90).

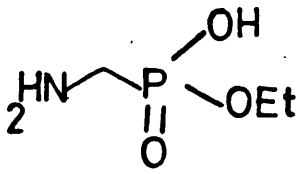


(90)



(91)

Subsequently Medved[89,90,91] used diethylhalomethylphosphonates to prepare the phosphonic analogue of glycine (92).

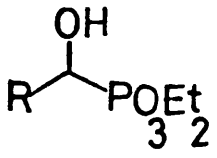


(92)

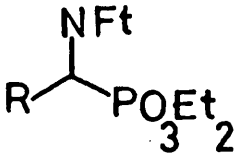
Reaction of chloromethylphosphonic acid with primary amines in the presence of sodium hydroxide was employed to obtain α-aminophosphonic acid derivatives for use as chelating agents[92,93]. However, these methods did not yield products in which the substituents on the amino group could be easily removed.

6. Hydroxymethyl Phosphorus Precursors.

The Mitsunubo reagent was utilised in a mild conversion of α-hydroxyphosphonates (93) to α-phthalimidophosphonates (94)[94]. A useful synthesis of α-phthalimidophosphonates has recently been described[95].



(93)

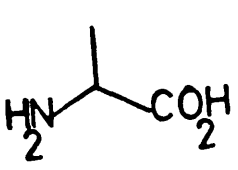
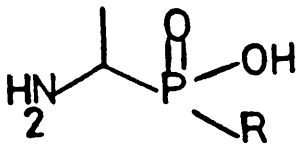
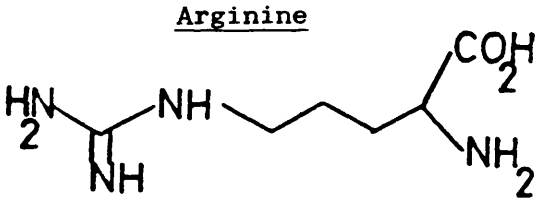
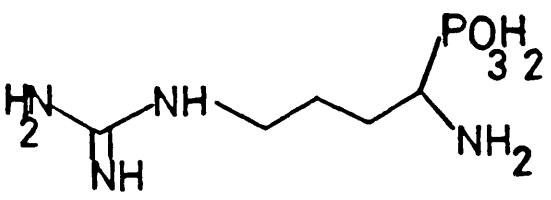
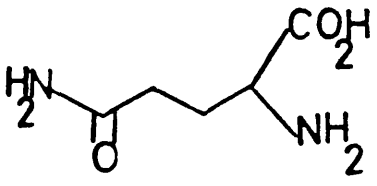
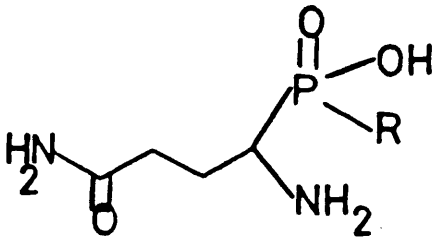
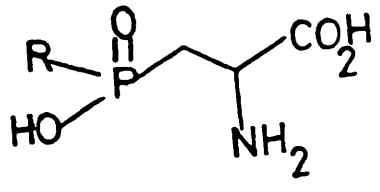
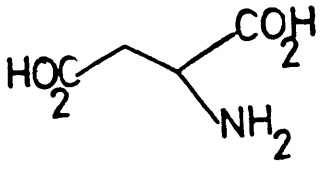
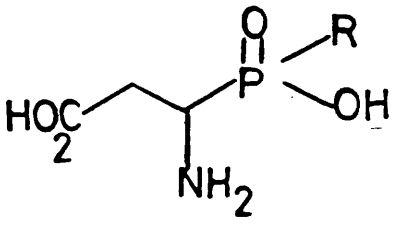
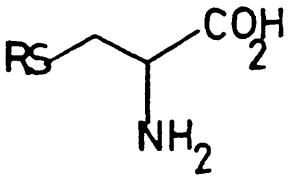
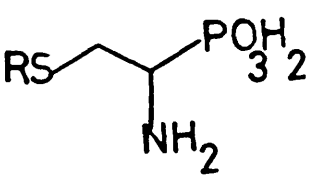
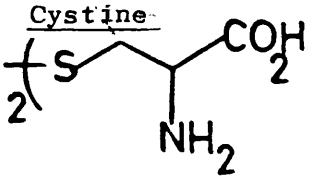
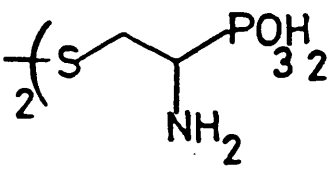


(94)

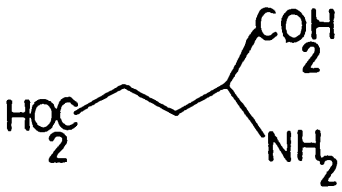
III α-Aminophosphonic and α-Aminophosphinic Acid analogues of the naturally occurring α-amino acids.

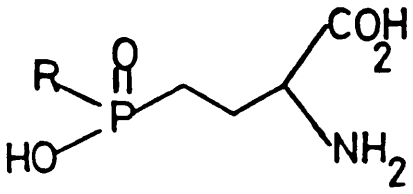
The α-aminophosphonic and α-aminophosphinic acid analogues of the naturally occurring α-amino acids that have been synthesised to date are represented in Table 1.

Table 1.

α-Amino acid	Phosphonic/Phosphinic acid analogues	References
Alanine	 	6, 17, 39, 50 53, 69, 46, 108.
Arginine	 	96
Asparagine	 	41 , 54, 65
Aspartic acid	  	53, 54, 54
Cysteine	 	82, 83
Cystine	 	82

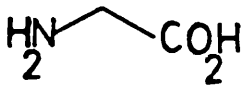
Glutamic acid

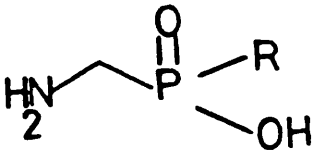




53, 97, 98, 99, 100, 101.

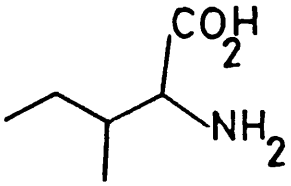
Glycine

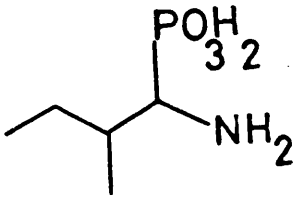




6, 53, 77, 89, 102, 103, 46

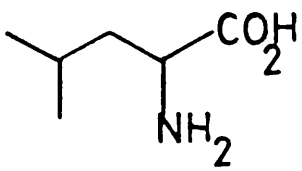
Isoleucine

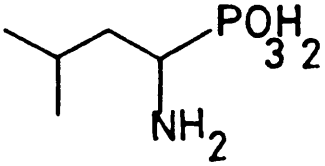




50, 51, 104

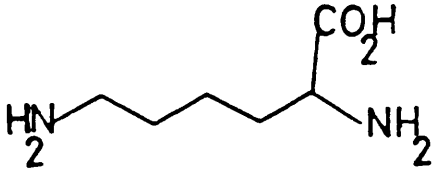
Leucine

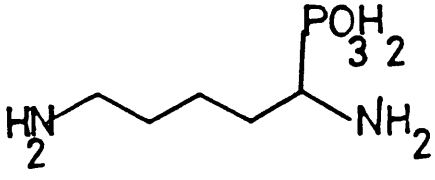




50, 51, 104, 106.

Lysine

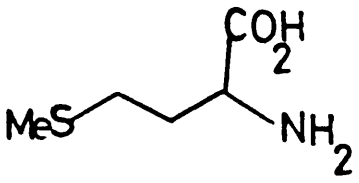


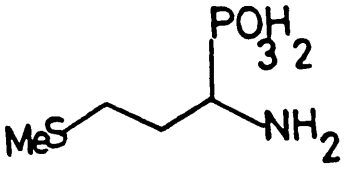


105

Methionine

85, 107

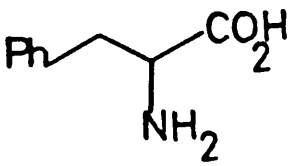


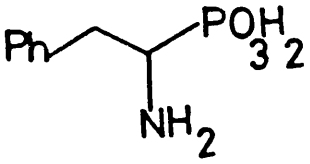


Phenylalanine

6, 40*, 53

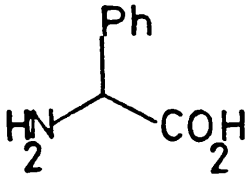
106, 57, 81

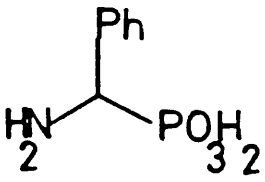




Phenylglycine

106

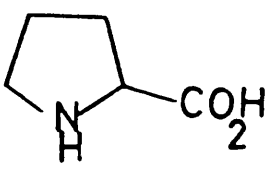


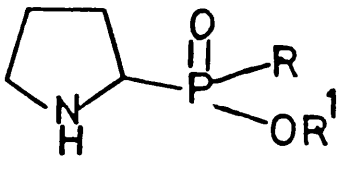


Proline

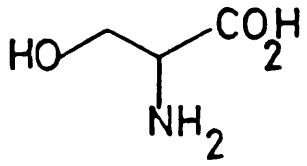
27, 70, 113,

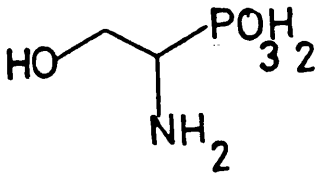
108





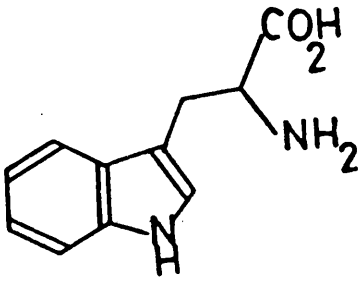
Serine

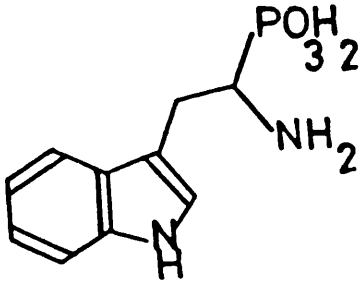




109

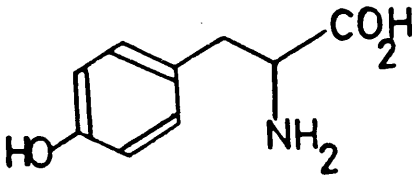
Tryptophan

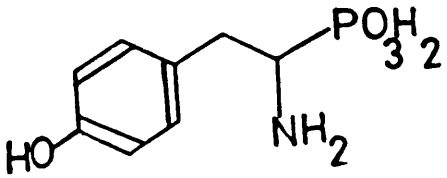




65, 107

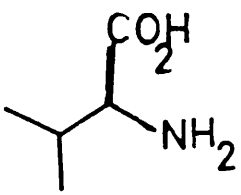
Tyrosine

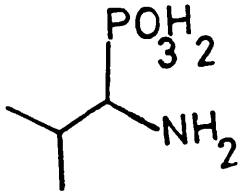




105

Valine





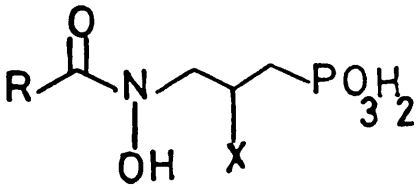
50, 51, 52, 68, 81, 87, 110, 111, 106

IV Phosphonic and Phosphinic Acids of Biological Interest.

Many phosphonic and phosphinic acids of natural and synthetic origin have been shown to exhibit biological activity. They may act as antibiotics, transition state analogues and enzyme inhibitors. Some of the more important phosphonic and phosphinic acids of biological interest are discussed.

A. Phosphonic acid antibiotics

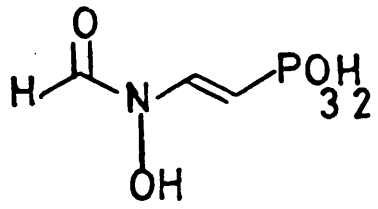
The isolation, structure elucidation and antibacterial properties of five new phosphonic acids antibiotics were described by Fujisawa Laboratories[114]. FR-900098 (95), FR-31564 (96), FR-33298 (97), FR-32863 (98) and FR-900137 (99) were isolated from culture broths of Streptomyces.



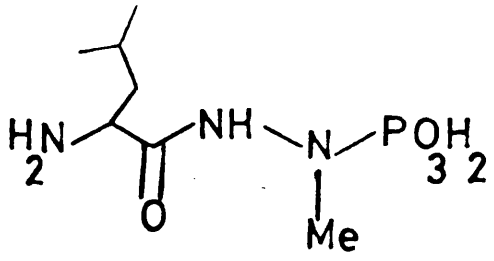
(95) R = Me, X = H

(96) R = H, X = H

(97) R = Me, X = OH



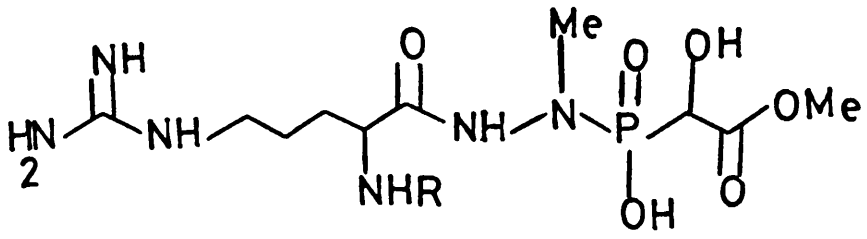
(98)



(99)

The activity of all five antibiotics was associated with cell wall growth. Compound (99) exhibited Gram positive and negative activity whereas compounds (95) to (98) exhibited broad spectrum

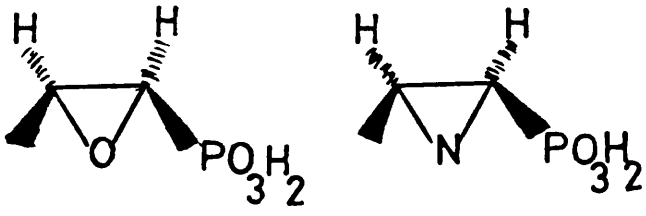
Gram negative activity. Syntheses of (95) to (98) have been reported[115]. The phosphazacins A and B (100) and (101)[116] have unique structures, antibiotic activity and resemble the Fujisawa antibiotics described above.



(100) R = H

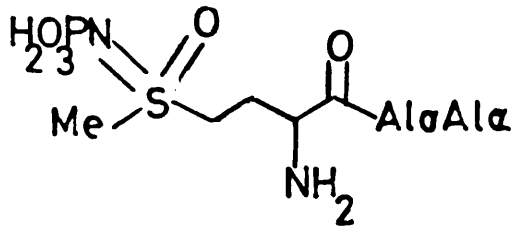
(101) R = $COCH(NH_2)iPr$

Phosphonomycin (102)[117] (-)(1R, 2S)-1,2-epoxypropylphosphonic acid) isolated from Streptomyces fradia was active against gram positive and gram negative bacteria. Its mode of action involved irreversible binding to the enzyme pyruvate uridine diphospho-N-acetyl glucosamine, thus inhibiting cell wall biosynthesis. The analogue (103) an aziridinylphosphonic acid[118] was also thought to possess antimicrobial activity.



(102) (103)

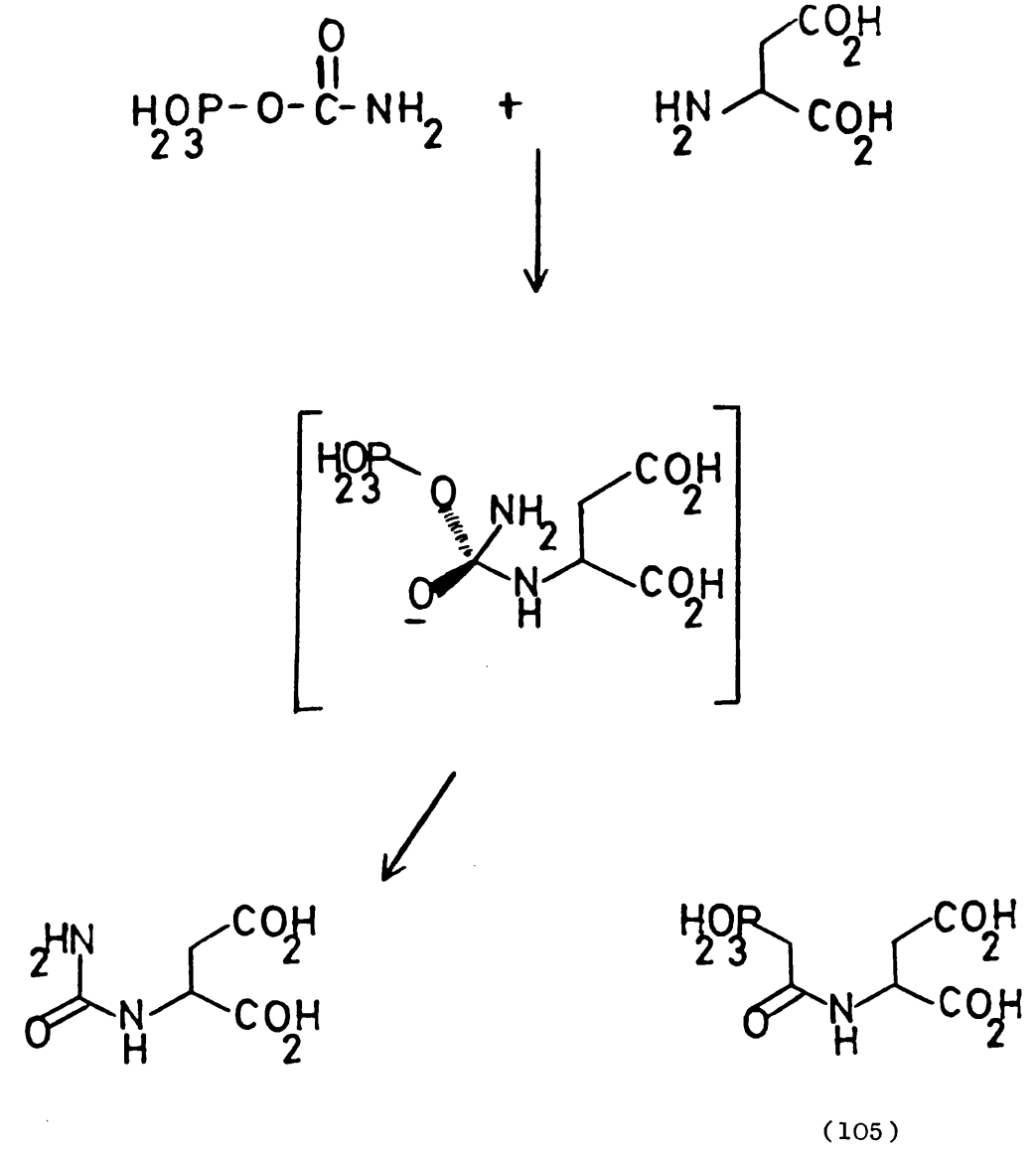
L-(N^5-Phosphono)-methionine-S-sulphoximinyl-L-alanyl-L-alanine (104) isolated at Hoffman-La Roche[119] from a Streptomyces species was active against gram positive and gram negative bacteria. Its activity was thought to be due to glutamine synthetase inhibition.



(104)

B. Other biologically active phosphonic and phosphinic acids

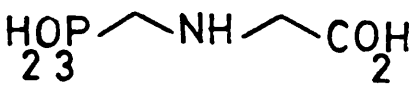
N-Phosphonoacetyl-L-aspartate (PALA) (105, Scheme 30) is a transition state analogue for aspartate transcarbamylase. Its synthesis[120] was rationalised on the basis that a stable surrogate for the transition state could be a specific and potent enzyme inhibitor. It was found that (105) bound to aspartate transcarbamylase one thousand times more strongly than carbamyl phosphate. PALA (105) has shown considerable activity against certain transplantable tumours in mice[121] and was shown to be a potent specific inhibitor of de novo pyrimidine synthesis.



(105)

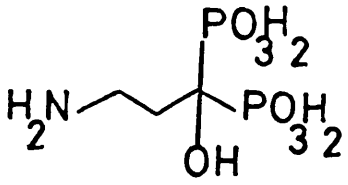
(Scheme 30).

Glyphosate[122], N-(phosphonomethyl)glycine (106) is a unique post-emergence herbicide. Its mode of action[123,124] is due to inhibition of the biosynthesis of aromatic amino acids.



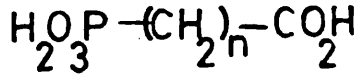
(106)

APD[125], 3-Amino-1-hydroxypropyl-1,1-diphosphonic acid (107) has found application in the treatment of Paget's disease, a chronic, progressive disorder characterized by bone softening and increased skeletal deformation.



(107)

Phosphonoacyl compounds active against herpes simplex virus have been produced. Phosphonoformate (PFA), phosphonoacetate (PAA) and phosphonopropionate (PPA) (108) are less highly cytotoxic than the standard antiviral compounds idoxuridine and vidarabine. They are competitive inhibitors of pyrophosphate during the herpes virus induced DNA polymerase reaction, and bind to the enzyme at the pyrophosphate binding site[126].



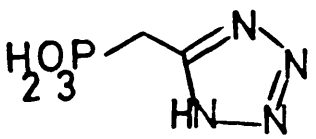
(108)

n = 0, PFA

n = 1, PAA

n = 2, PPA

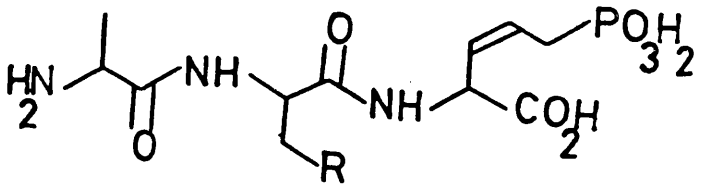
The tetrazole analogue (109)[127] showed greater inhibition of herpes simplex II than PAA, but lower inhibition of type I than PAA.



(109)

V. Biologically Active peptides with a terminal Phosphonic or phosphinic acid

Plumbemycins A and B (110) were isolated from Streptomyces plumbeus[128] and assigned L-alanyl-L-aspartyl-D-2-amino-5-phosphono-3-cis-pentanoic acid and L-alanyl-L-asparaginyl-D-2-amino-5-phosphono-3-cis-pentanoic acid respectively. Both antibiotics are threonine antagonists and are transported into bacterial cells via the oligopeptide transport system[129].

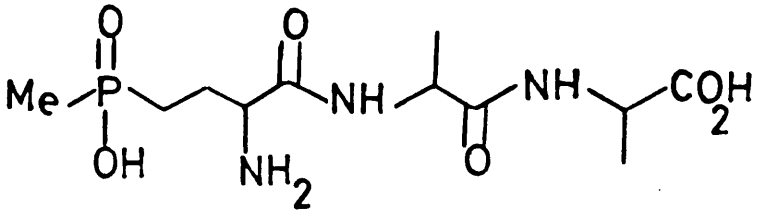


(110)

R = CO_2H, Plumbemycin A.

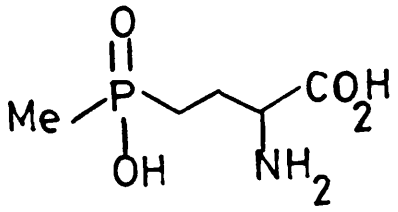
R = $CONH_2$, Plumbemycin B

The antibiotic Phosphinothricin, Phosphinothricyl-alanyl-alanine (111) was isolated from Streptomyces hygroscopicus[130] and Streptomyces viridochromogenes[131]. It is highly active against gram positive and gram negative bacteria and is a possible glutamine synthetase inhibitor[129], entering via the olig opeptide system.

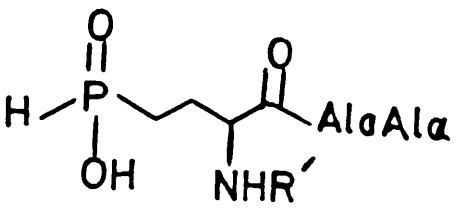


(111)

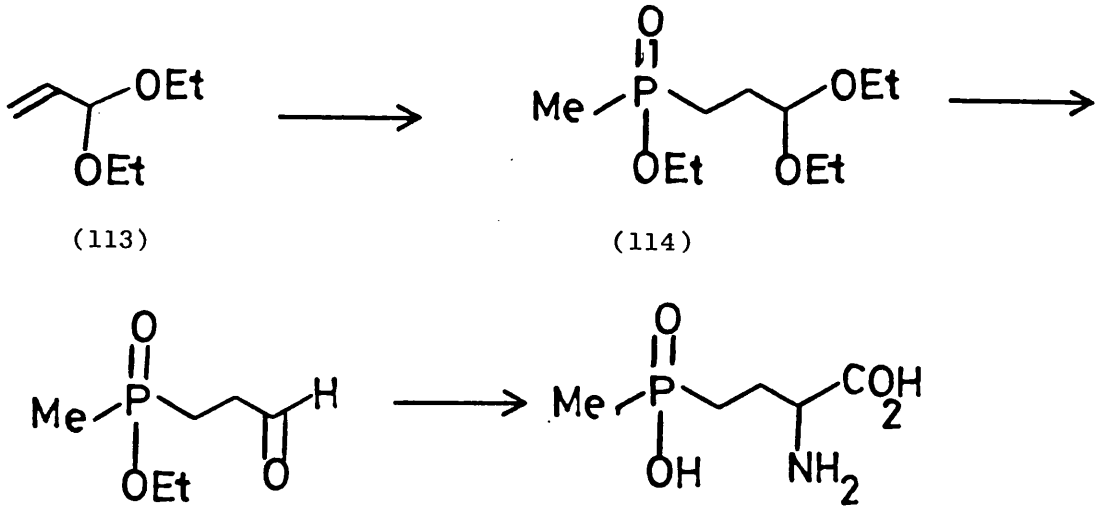
The novel, 2-amino-4-methylphosphinobutanoic acid component (112) of the tripeptide (111) is itself a herbicide, which possibly inhibits glutamine synthetase. It was synthesised[131] (Scheme 31) by radical addition of a methylphosphonite to acrolein acetal (113) followed by hydrolysis of (114) and Strecker reaction of (115). Other syntheses started from homoserine[131], acetylaminomalonate[132] and from α-propionaldehyde acetals[133]. A recent and different synthetic strategy (Scheme 32) employed a vinyl phosphonate which was transformed via a Michael reaction to (±)-phosphinothricin in good yield[134]. The isolation of bialaphos (116), which is structurally related to phosphinothricin, has been described.



(112)



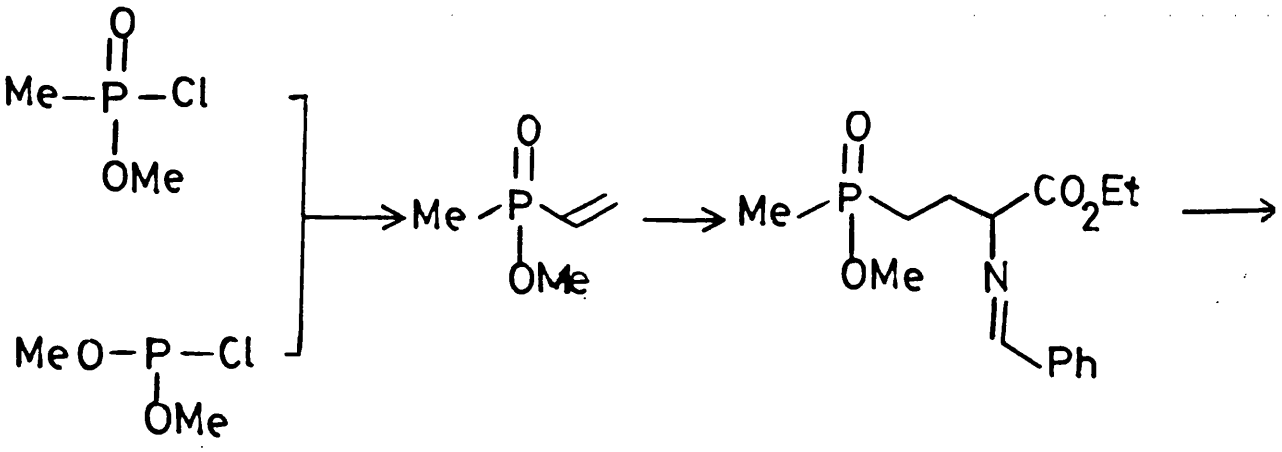
(116)



(113) (114)

(115) (116)

(Scheme 31).



(112)

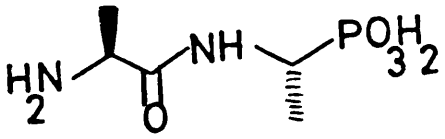
(Scheme 32).

Alaphosphin[135] (L-alanyl-L-1-aminoethylphosphonic acid) (117) is a synthetic antibacterial agent prepared by Roche, designed to mimic the terminal peptide moiety (D-alanyl-D-alanine) of the peptidoglycan involved in bacterial cell wall biosynthesis. The mode of action is thought to involve three stages[136]:-

1. transport through the bacterial cell wall by peptide permeases;

2. intracellular peptidase cleavage; and

3. action of the L-1-aminoethylphosphonic acid on alanine racemase.

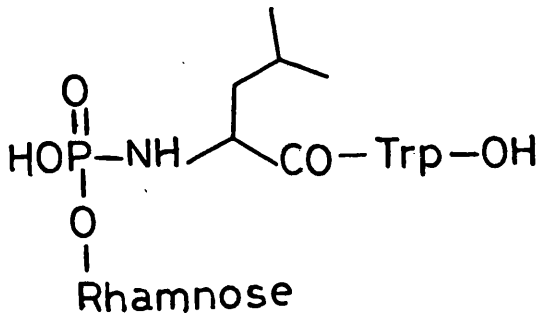


(117)

Alaphosphin has greater effect against gram negative than gram positive bacteria. A range of derived and related phosphonopeptides have also been described[137].

VI Peptides containing a phosphoramidate moiety

Phosphoramidon, N-(α -L-rhamnopyranosyloxyhydroxy-phosphinyl)-L-leucyl-L-tryptophan) (118) has been isolated from Streptomyces tanashiensis and shown to be a potent inhibitor of the thermostable metalloprotease thermolysin[138]. Fig. 1 shows a schematic representation of the interactions observed in the phosphoramidon-thermolysin complex[139]. A series of N-phosphorylated dipeptides and amino-acid derivatives were synthesised[140]. These compounds which are analogues of phosphoramidon were shown to be competitive inhibitors of thermolysin,



(118)

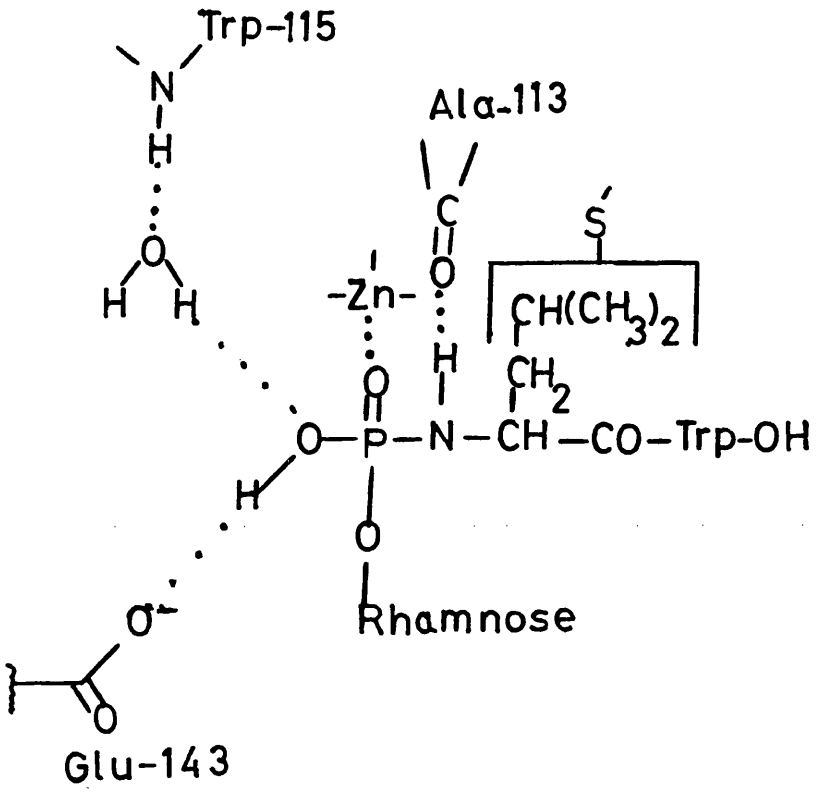

Trp-115
N
H
O
H
H
Ala-113
C
O
S
-Zn-
CH(CH3)2
O
H
CH2
O—P—N—CH—CO—Trp-OH
H
O
Rhamnose
O
O
Glu-143

Fig. 1.

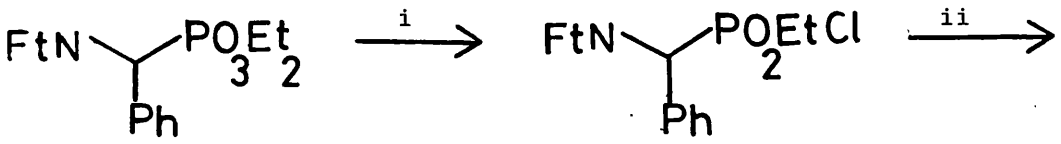
VII Endophosphonopeptides:- Peptide Analogues containing a phosphonamide bond

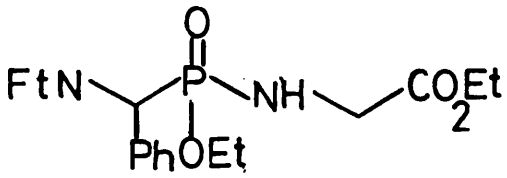
Dipeptide analogues linked with a phosphonamide bond were produced by the reaction of C-protected amino acids with N-protected aminophosphonomonochloridates[141,142,143].

Japanese workers[141] and others[142] coupled N-phthaloyl aminophosphonomonochloridates with amino acid esters, producing the dipeptide analogues (119) and (120) (Scheme 33). The Japanese group extended this methodology leading to a synthesis of tripeptides containing aminomethylphosphonic acid[141,142]. No biological data on these protected peptide analogues was reported.

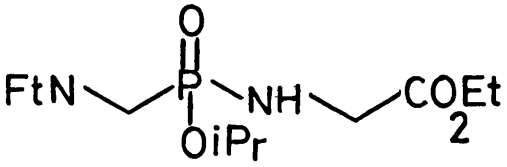
Recently the activation of N-protected aminophosphonic acids using diphenylphosphorylazide (DPPA) was described in the synthesis of peptide analogues containing (2-Aminoethyl) phosphonic acid[145].

Bartlett[143] synthesised the endophosphonodipeptide (121) by coupling the N-benzyloxycarbonyl protected aminophosphono-monochloridate with phenylalanine methyl ester (Scheme 34).





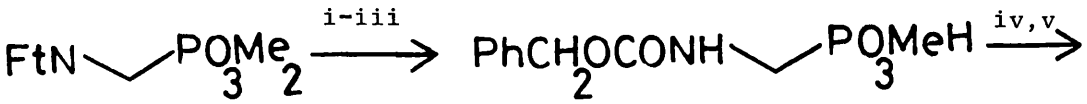
(119)

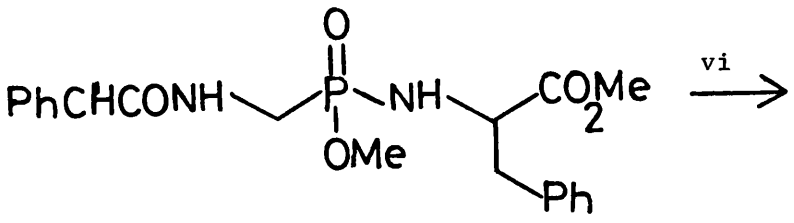


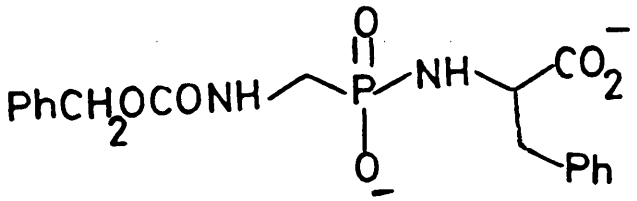
(120)

i, PCl_5; ii, glycine ethyl ester

(Scheme 33).







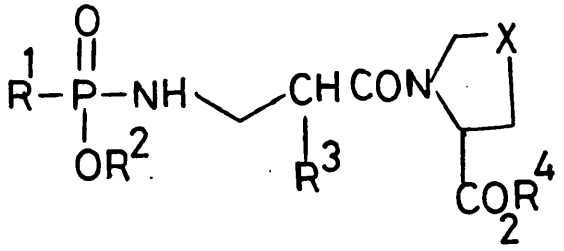
(121)

i, H_2NNH_2; ii, $PhCH_2OCOCl$; iii, NaOH; iv, $SOCl_2$, v, phenylalanine methyl ester; vi, LiOH.

(Scheme 34).

Bartlett[143] also devised a novel strategy for the inhibition of carboxypeptidase A (CPA). The endophosphonodipeptide (121) may be regarded as a transition state surrogate for the tetrahedral intermediate in the proposed mechanism for CPA-catalysed peptide hydrolysis. Thus (121), an analogue for the transition state of z-Gly-L-Phe, having a phosphonamidate moiety in place of the scissile peptide group, was shown to be a competitive, reversible inhibitor of CPA.

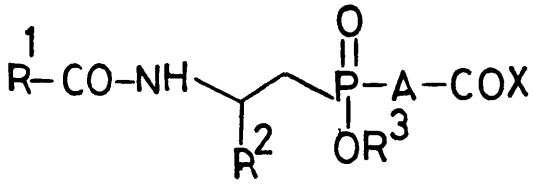
Merck[146] have synthesised phosphonamide (122) and related compounds as potential angiotensin converting enzyme (ACE) inhibitors and antihypertensives.



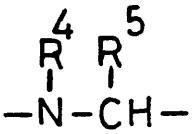
(122)

wherein R_1 = acylamino

Squibb[147] have also examined phosphosponamides (123) for the same reason.



(123)

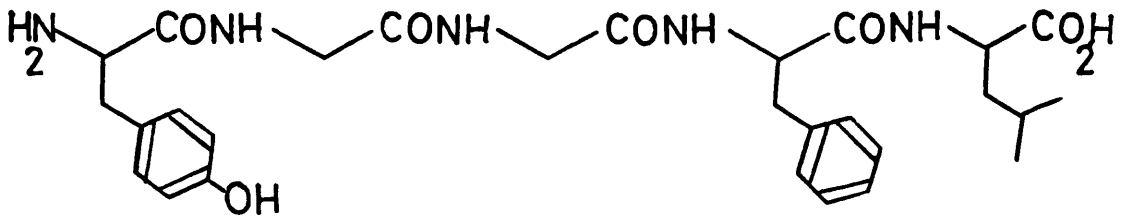
wherein A is 

Compounds of general formula (122) and (123) were synthesised *via* the corresponding N-protected aminophosphonomonochloridate.

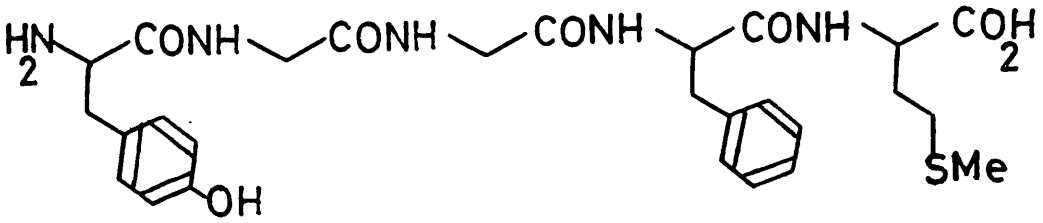
VIII The Enkephalins

In the early 1970's Liebeskind and his associates were able to demonstrate that electrical stimulation of specific brain sites in the rat produced profound analgesia[148] which was naloxone reversible[149], and subject to tolerance development and to cross-tolerance to morphine[150]. These results were explained by the electrically induced release of an endogenous substance or substances with morphine-like properties. Close correlation was also observed between brain areas most sensitive to stimulation-produced analgesia and regions containing a high density of opiate receptors.

Early in 1975 evidence emerged that these endogenous opioids were peptides rather than simple morphine-like molecules[151]. Extracts from pig brain provided in 1975 by John Hughes showed these endogenous opiods to be two peptides[152], each with five amino acids and differing only in the carboxyl terminal amino acid. (Fig. 2). They christened them methionine (Met) and Leucine (Leu) enkephalin, the term enkephalin being coined from the Greek word meaning "in the head". The enkephalins appear throughout the central nervous system and gastrointestinal tract[153].



Leu-enkephalin



Met-enkephalin

Fig.2. Methionine and Leucine enkephalin

Evidence has shown that enkephalins function at the neuronal level as presynaptic neurotransmitters[154]. They are concentrated in nerve terminals and are released by depolarising stimuli[155].

The opiate receptor sites that have been most widely studied are those of the Guinea Pig ilieum (GPI) and Mouse Vas deferens (MVD) tissue preparations. The receptors at these

sites are called μ and δ respectively. Initial studies on ^{3}H-enkephalin binding to opiate receptors revealed a different order of relative potencies for certain opiates than that previously seen under ^{3}H-opiates. In addition the enkephalins were far less sensitive to the effects of sodium ion on binding affinity. Lord and Kosterlitz also found that Met- and Leu-enkephalin demonstrated an enhancement of (MVD) activity over (GPI) activity[155,156]. This led them to suggest that the enkephalins interact more specifically at the δ-opiate receptors.

More recently a new opiate-receptor has been discovered called kappa (κ). These receptors can be found in high concentration in the Rat Vas deferens (RVD). The Table 2 shows the different types of opiate receptor and summarises the effect of interaction at that receptor site.

Table 2.

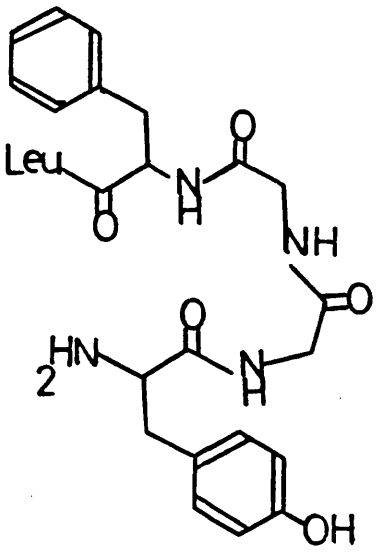
μ	δ	κ
Analgesia	Analgesia	Analgesia
Addiction	Respiratory	Sedation
Euphoria	Depression	Dysphoria
Constipation		
Sedation		

The heterogeneity or binding sites (μ , δ , κ ....) would explain the wide range of pharmacological effects elicited by administration of non-discriminant drugs such as morphine. With the concepts of receptor multiplicity detailed considerations of opiod structure reactivity relationships have been undertaken to prepare new molecules exhibiting the highest possible specificity for each kind of receptor and thereby reduce any unnecessary side effects.

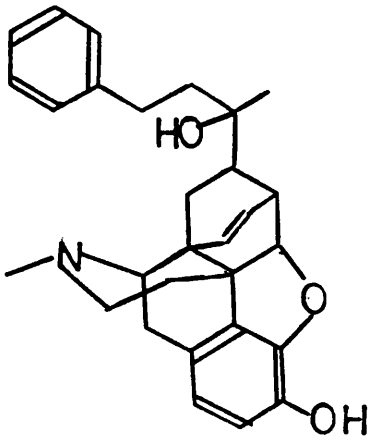
A. Identification of functional Groups Required for Biological Activity in the Enkephalins

Early structure-activity data for the enkephalins demonstrated that 1) the phenolic group of Tyr^1 was important for activity[157,158] and 2) (desamino-Tyr^1) Met-enkephalin was devoid of biological activity[159]. It has also been shown that the appropriate spatial conformation is available for the phenolic ring and quaternary nitrogen present in the phenanthracene nucleus of opiate alkaloids and Tyr^1 of the enkephalins[160]. These observations indicated that Tyr^1 of the enkephalins provided two sites for binding at the opiate receptor. The necessity of Phe^4 was also shown for enkephalin recognition[161,162] and several investigators have related the aromatic side chain of Phe^4 with the 19-Phenethyl substituent (F ring) of the opiate compound 7-(1-phenyl-3-hydroxybutyl)-3-endoethenotetrahydrothebaine (P.E.T.)[163]. These observations have been confirmed and novel analogues of enkephalins have been prepared to identify the functional groups required for biological activity[166]. The effect of structural change at each amino acid position has been studied using either Met-enkephalin, Leu-

enkephalin or a more stable enkephalin analogue[167].

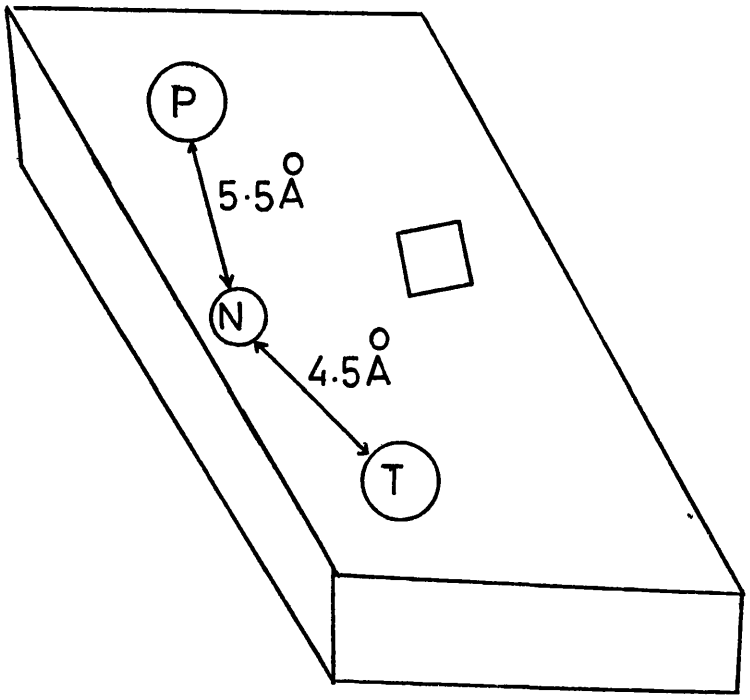


Leu-enkephalin



P.E.T.

A simplistic model of a receptor site is shown where one aromatic subsite (T-site) preferentially binds the hydroxylated rings (as in morphine and tyrosine in enkephalin). The anionic site is $\approx$ 4.5Å from the centre of the ring to the piperidine nitrogen. The second aromatic site and the site that binds the non-hydrosylated flexible equatorial aromatic ring such as mepherdrine or the phenylalanine in enkephalin. The steric hump interacts with substituents at C14 (morphine) to elevate the nitrogen from the anionic site thereby confering antagonistic properties.



B. Enkephalin Analogues Containing aminophosphonic and aminosulphonic acid residues at the C-terminus

Bajusz replaced the residue at position five of the enkephalins with an appropriate aminophosphonic or amino sulphonic acid[168]. The opiate agonist activities determined in MVD and GPI tissue preparations are summarised in table 3.

Generally higher potencies were obtained in the MVD than the GPI. Thus the bulky, dibasic $-PO_3H_2$ group at the C-terminus seems to be preferred or tolerated more by the δ-receptors of the MVD rather than the μ-receptors of the GPI.

Table 3.

Opioid agonist activities of enkephalin analogs Tyr-Xxx-Gly-Yyy in MVD and in GPI given in $10^{-6}/IC_{50}$ and their analgesic potencies relative to morphine (=100)

Tyr-Xxx-Gly-Phe-Yyy	MVD $10^{-6}/IC_{50}$[a]	GPI $10^{-6}/IC_{50}$[a]	$\frac{\text{MVD}}{\text{GPI}}$	analgesic potency[b]	
				i.c.v.	i.v.
——Gly————Nle	48.49(1)[c]	2.74(1)[c]	17.7	-	-
——Gly————NleS	235.89(4.8)	16.99(6.2)	13.9	2	2
——Gly————NleP	430.96(8.9)	7.81(2.8)	55.2	1	1
——Gly————D-NleS	5.48(0.1)	0.27(0.1)	20.3	2	2
——D-Ala————Nle	1000.00(1)	15.29(1)	65.4	-	-
——D-Ala————NleS	1786.03(1.8)	42.81(2.8)	41.7	2.0	2
——D-Ala————NleP[d]	5130.84(5.1)	61.16(4.0)	83.9	15.0	2
——D-Ala————D-N-eS[d]	58.10(0.06)	15.29(1.0)	3.8	9.8	2
——D-Nle————Nle	1077.24(1)	16.78(1)	64.2	-	-
——D-Nle————NleS[d]	4761.90(4.4)	117.45(7.0)	40.5	2	2
——D-Nle————NleP	4221.19(3.9)	26.84(1.6)	157.3	4.5	2
——D-Nle————D-NleS	10.07(0.01)	8.39(0.5)	1.2	2	2

Table 3 (contd.)

Tyr-Xxx-Gly-Phe-Yyy	MVD $10^{-6}/IC_{50}$[a]	GPI $10^{-6}/IC_{50}$[a]	MVD / GPI	analgesic potency[b]	
				i.c.v.	i.v.
——D-Met———Nle[d]	1667.78(1)	25.97(1)	64.2	-	-
——D-Met———NleS	2041.65(1.2)	54.54(2.1)	40.5	9.5	2
——D-Met———NleP	618.20(0.4)	9.35(0.4)	66.1	25.0	2
——D-Met———D-NleS	5.19(0.003)	10.39(0.4)	0.5	6.4	2
——D-Met———D-NleP	7.53(0.004)	10.65(0.4)	0.7	52.0	16.8

[a] Dimension is M^{-1}

[b] ED_{50} values of morphine in the tail-flick test in rats were 3.14 nmol/animal after intracerebroventricular (i.c.v.) administration and 1.9 mol/kg after intravenous injection (i.v.)

[c] Changes in activity relative to the parent Nle^5 analogs

[d] The most active analogs of Nle^5-$NleS^5$-, D-$NleS^5$-and $NleP^5$-enkephalins

IX Conclusion

Many peptide analogues containing phosphorus have been synthesised and biologically assessed. The molecules most studied have C-terminal phosphonic or phosphinic acids or contain phosphoramide or phosphonate moieties.

Peptide analogues containing phosphonamide bonds have received little attention. The synthesis of an analogue of an endogenous peptide with a phosphonamide link has never been reported.

The synthesis of an endophosphonoenkephalin will be discussed with the aim to producing:

1) a substrate that may bind to an enzyme forming a stable transition state surrogate

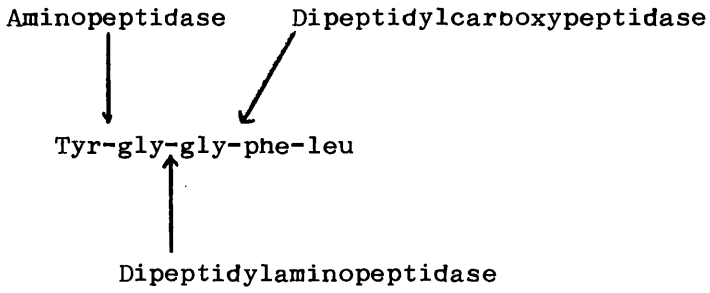
2) an enkephalin analogue that may exhibit opoid agonist activity.

DISCUSSION

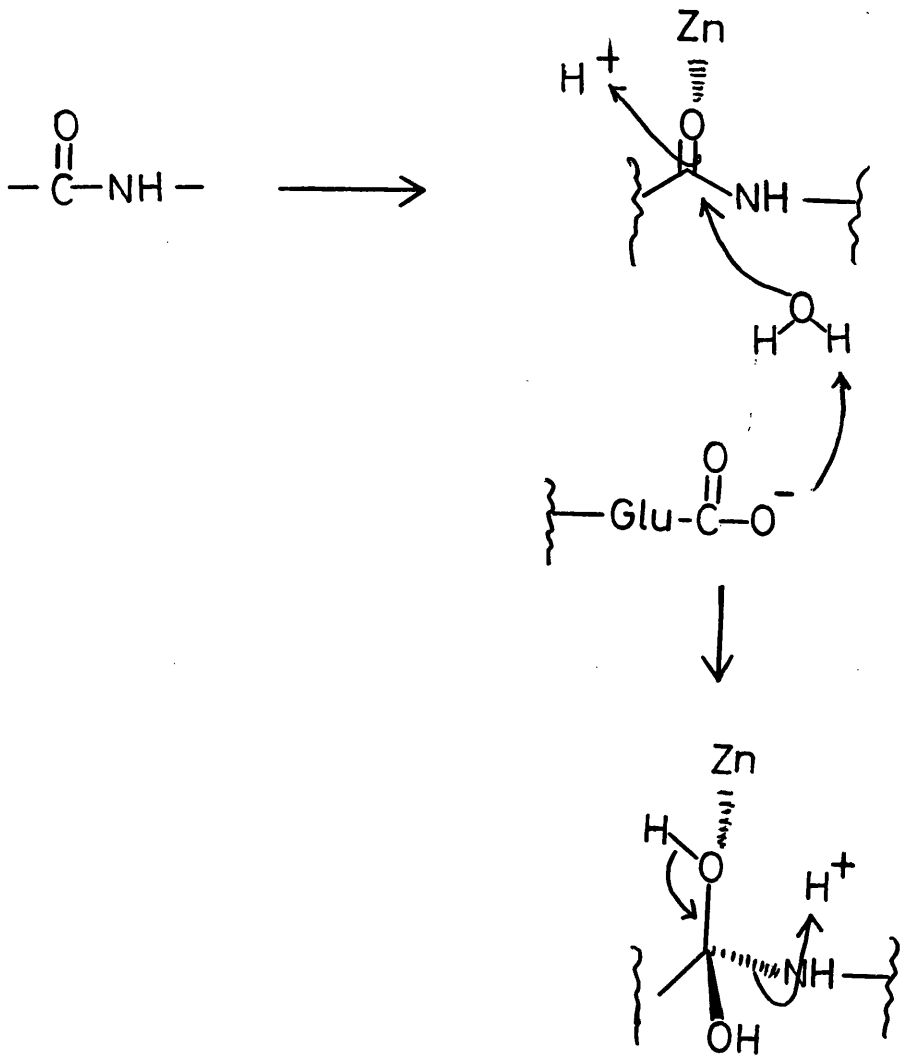
Introduction

1. Modes of Peptide hydrolysis by carboxypeptidase

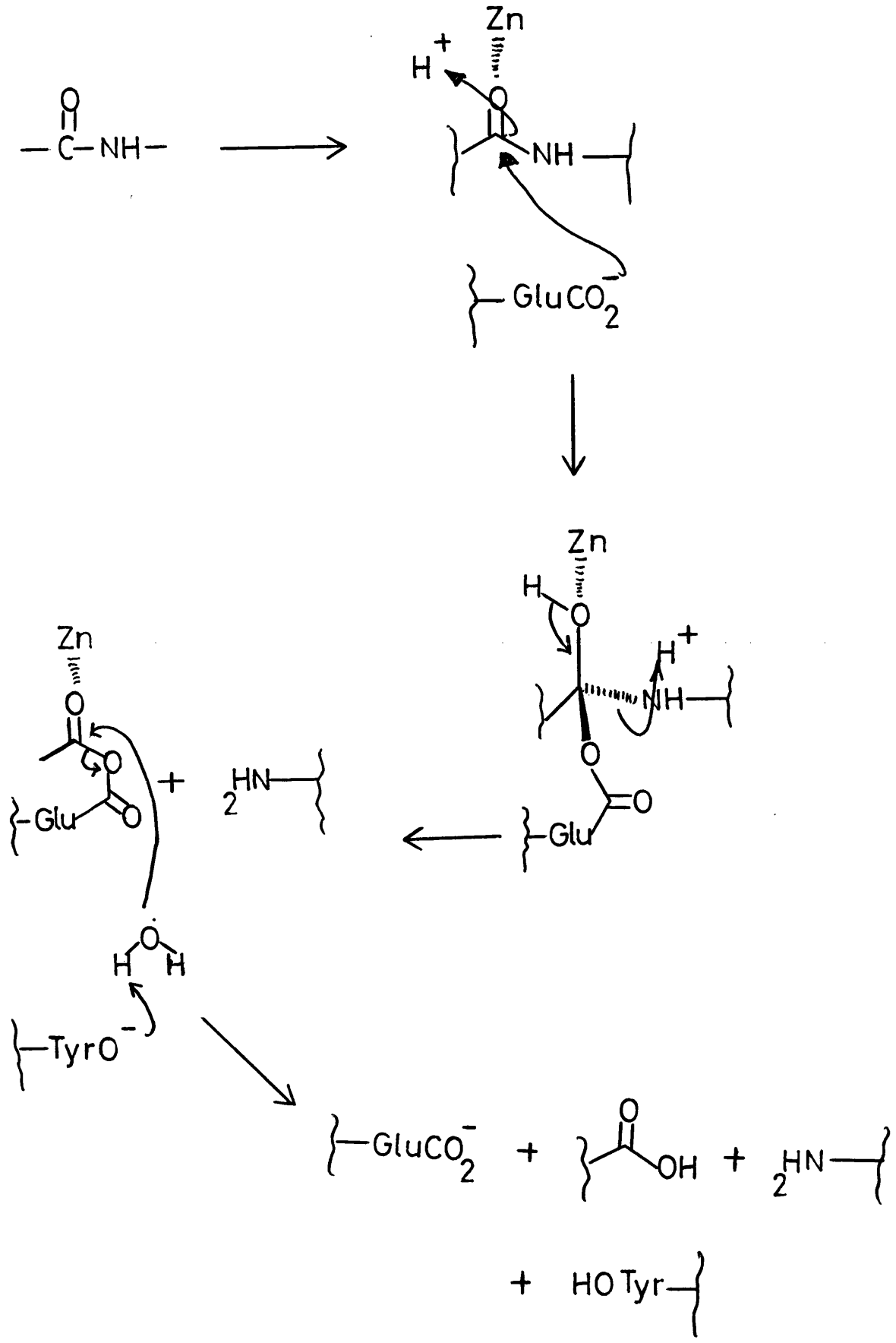
All but one of the amide bonds in Leu and Met enkephalin may be hydrolysed enzymatically[169]. The sites of attack are illustrated below:



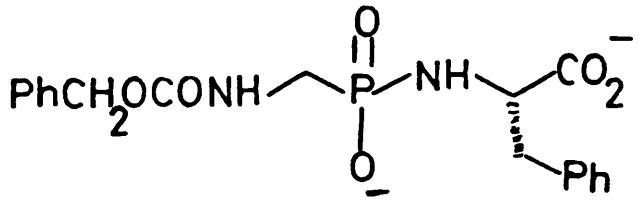
The zinc peptidases constitute an important class of proteolytic enzymes, from both the mechanistic and biological standpoint[170]. Carboxypeptidase A (CPA) is the leading representative of this group. It is specific for the cleavage of C-terminal amino-acids from oligopeptides, favouring those with aromatic side chains. X-ray crystallography has defined the configuration of the enzyme active site[171], and has led to widely accepted mechanisms of peptide hydrolysis by zinc containing enzymes[172]. The two probable mechanisms both of which involve a tetrahedral intermediate are shown below.


Zn
H
O
NH
H
O
H
Glu-C-O
Zn
H
O
H
NH
OH


COH + HN
GluCOH


Zn
H
NH
GluCO2
Zn
H
H
NH
Glu
Zn
Glu
HN2
TyrO
GluCO2
OH
HN2
HO Tyr

Bartlett's interest in tetrahedral phosphorus derivatives as stable mimics of tetrahedral carbon intermediates[174] led to the preparation of a phosphonamide - analogue of a CPA substrate, carbobenzoxyglycyl-L-phenylalanine. This analogue N- [[[(benzyloxycarbonyl)amino]methyl]hydroxyphosphinyl]-L-phenylalanine, dilithium salt(121) was designed to resemble the tetrahedral adduct formed during peptide hydrolysis. This was the first phosphonamide employed as an enzyme inhibitor[143].



(121)

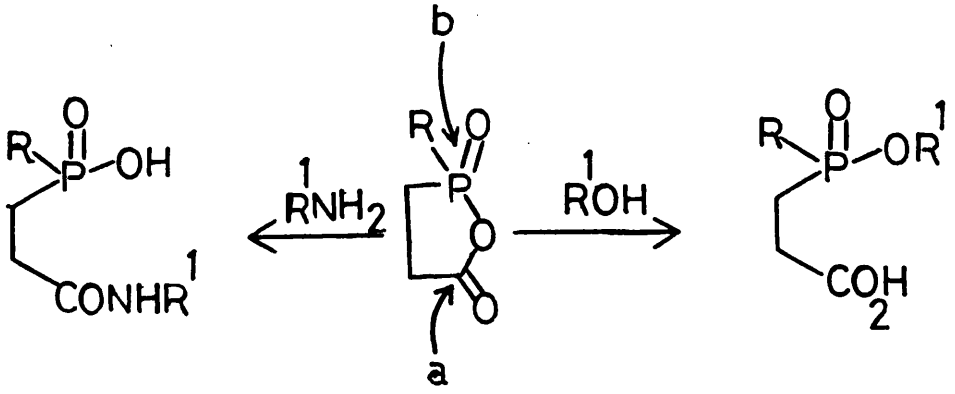
Bearing in mind the sites of enkephalinase attack, an enkephalin analogue containing a phosphonamide bond between gly-phe might well be expected to be an inhibitor of dipeptidyl carboxypeptidase "Enkephalinase A". This work describes our synthetic efforts towards the synthesis of an endophosphono enkephalin.

2. Evaluation of coupling methods for the formation of phosphonamide peptide analogues

a) Attempted coupling of aminophosphonic acids by the mixed anhydride method.

The coupling of aminophosphonic acids using dicyclohexylcarbodiimide (DCC) has been reported to be unsuccessful[142]. The use of carboxylic-phosphonic anhydride systems for the formation of phosphonamide has not been described.

Carboxylic-phosphinic anhydrides have not received much attention. However, some information concerning the cyclic system (124) was reported.[175]

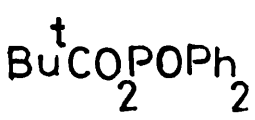


(124)

In this case an important change in regiospecificity depending on the nature of the nucleophile was observed. Aminolysis followed path(a) whereas alcoholysis occurred by attack at phosphorus.

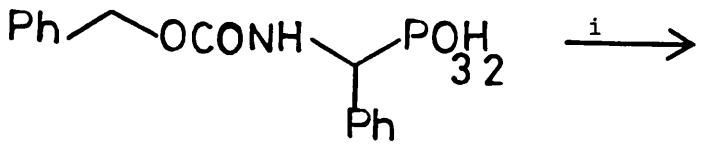
We investigated the activation of N-benzyloxycarbonyl phosphonophenylglycine using pivaloyl chloride. Although it was demonstrated[175] that amines were better nucleophiles for carbon than for phosphorus in the carboxylic-phosphinic anhydride (124), it was anticipated that in our case steric hindrance due to the t-butyl groups could reverse this discriminatory effect. In fact the only product isolated was (125) due to attack at carbon (Scheme 35). This compound was characterised by NMR and mass spectrometry.

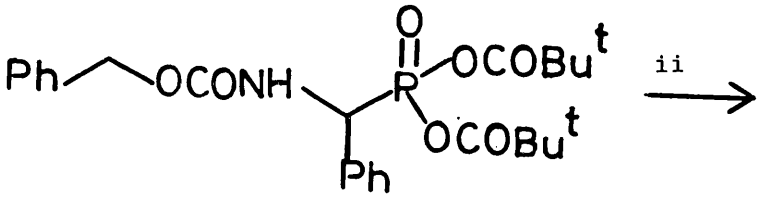
As a test of the relative merits of pivalic and diphenylphosphinic (DPP) mixed anhydrides compound (126) was prepared[175]. It was observed that (126) reacted with β -phenethylamine to give (127) exclusively.

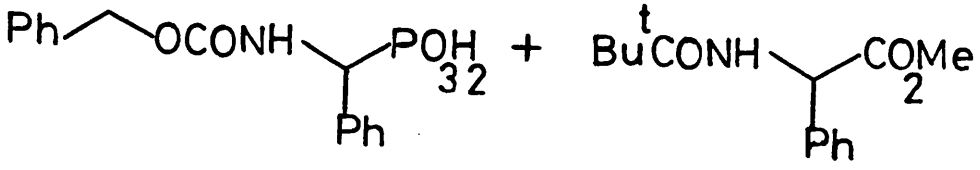


(126)

(127)







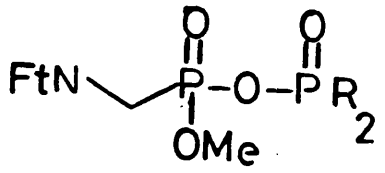
(125)

i, $(CH_3)_3COCl$, -23°C, Et_3N 2 eq; ii, Phenylglycine methyl ester.

(Scheme 35.)

Our studies concur with the reported observations[175] and demonstrate that mixed carboxylic-phosphinic anhydrides are not suitable for the formation of phosphonamides (Scheme 35).

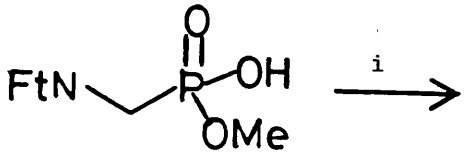
We also studied phosphonic-phosphonic and phosphonic-phosphinic anhydrides of type (128). These have also proved unsuccessful in phosphonamide formation.



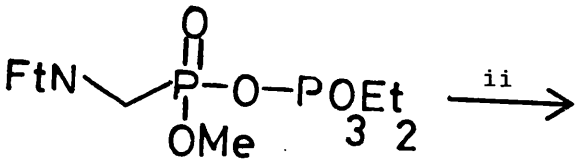
R = OPh, Ph, OEt

(128)

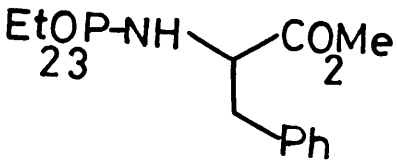
When R=Ph, OPh, no coupled products were isolated after hydrolytic work up including acid and base extraction. When R was OEt compound (129) was isolated (Scheme 36), the structure of which was clear from NMR data. The ^{1}H NMR spectrum showed a singlet at 3.78 due to the carbomethoxy ester and signals at 1.2 and 3.9 due to the phosphorus esters.



(135)



(130)



(129)

i, $(EtO)_2\overset{O}{\overset{\|}{P}}-Cl$, THF, $-20^{o}C$, NMM leq; ii, Phenylalanine methyl ester NMM 1 eq.

(Scheme 36.)

The formation of anhydride (130) was assumed in Step i when the precipitation of N-methyl morpholine hydrochloride was observed. Another explanation could be that the anhydride (130) had not formed and the phenylalanine methyl ester was reacting with unchanged diethyl chlorophosphate. From these studies it was concluded that phosphonic-phosphinic or phosphonic-phosphonic mixed anhydride systems were also not suitable for phosphonamide formation.

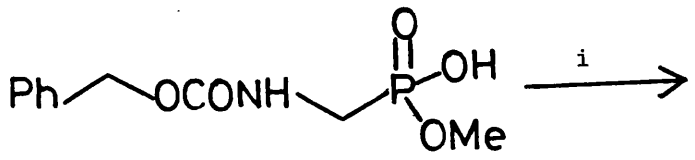
b) Coupling reactions of phosphonochloridates with C-protected amino acids and peptides

The formation of phosphonochloridates directly from phosphonate esters has been accomplished using phosphorus pentachloride[141]. However, as the reaction requires elevated temperatures, such as refluxing toluene, its use is relatively limited to molecules containing functional groups that can withstand the reaction conditions. The reaction has been employed successfully for N-phthaloyl protected aminoalkyl phosphonates[141,142].

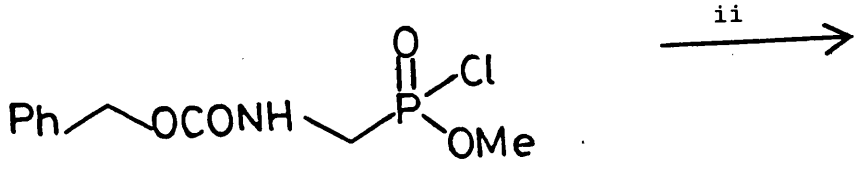
Our attempts to prepare phosphonochloridates directly from aminoalkylphosphonates with N-urethane and N-alkyl protection were unsuccessful.

Bartlett[143] reacted thionyl chloride with N-benzyloxycarbonyl

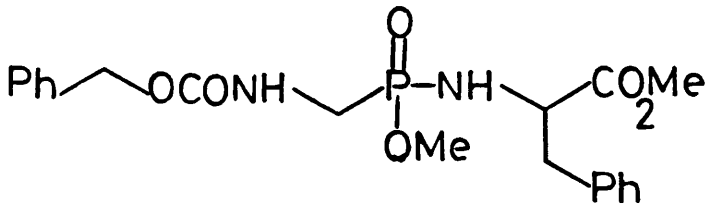
aminophosphonic acid (131) giving the N-protected aminophosphono-chloridate (132) which coupled successfully with phenylalanine methyl ester to yield the dipeptide analogue (133), Scheme 37.



(131)



(132)



(133)

i. $SOCl_2$; ii Phenylalanine methyl ester, Et_3N 1 eq.

(Scheme 37.)

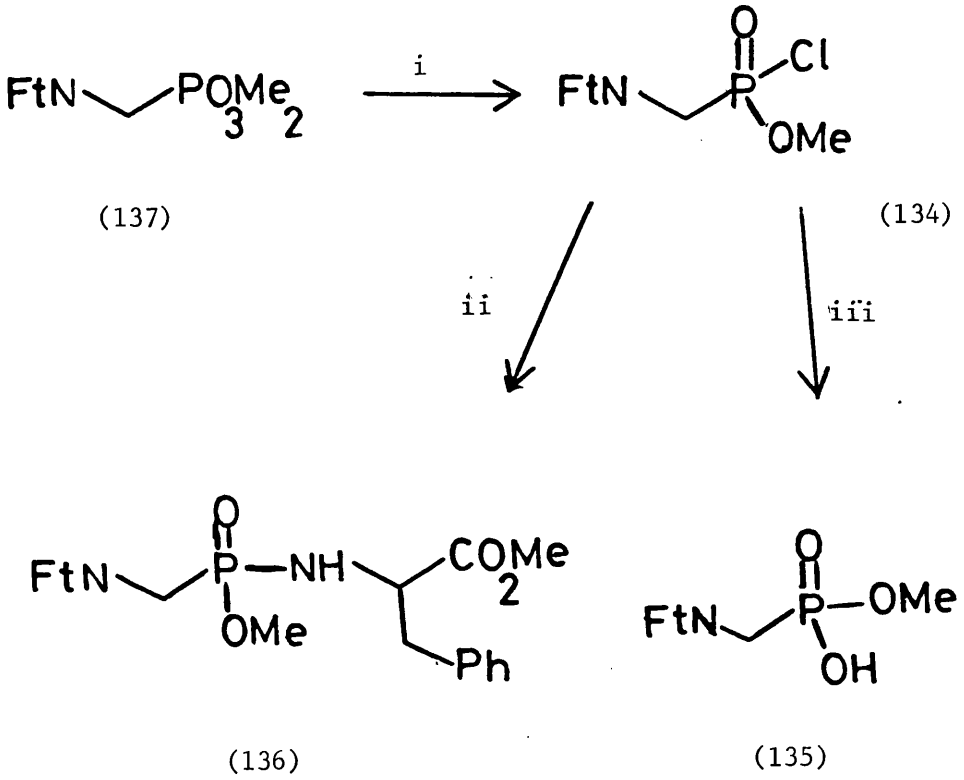
However, Barlett[143] synthesised (131) from the corresponding phthalimidomethyl dimethylphosphonate. This methodology was apparently chosen instead of utilising phosphorus pentachloride directly as removal of the N-phthaloyl functionality at a later stage may have proved impracticable. (See Section 4).

N-Phthaloyl protected endophosphonopeptides were prepared by Japanese workers and the N-phthaloyl group successfully removed using hydrazine[141,142]. Although the yields of these reactions were not very high the shorter route to phosphonamides compensated.

From the known methods available for phosphonamide formation from phosphonochloridate we decided to attempt to form the phosphonochloridates directly from N-phthaloyl protected aminoalkylphosphonates. This would enable us to employ the most economic route in terms of the number of steps into phosphonamide systems, and to investigate conditions for removal of phthalimide or related protecting groups.

We demonstrated the formation of the phosphonochloridate (134) by observing the downfield shift and change in coupling constant J_{P-H} of the methylene protons in (134) relative to the dimethyl ester. Upon deuteration of phosphonochloridate (134) the resonances due to the methylene protons occurred at higher field as compared to (134). After hydrolysis of (134) we isolated the novel monophosphonic acid (135) the structure of which was confirmed by NMR signals at δ7.85 (aromatic) 3.7-3.95 (CH_2P, J_{PH} 11Hz) and 3.5-3.75 ($P-OCH_3$, J_{PH} 11Hz). The IR spectrum showed stretches at 3400-2700 (P-OH), 1790, 1740 (C=O) and 1050 (P=O)cm^{-1}.

Having found suitable conditions for the formation of the phosphonochloridate (134) we synthesised the phosphonamide dipeptide analogue (136) as shown in Scheme 38.



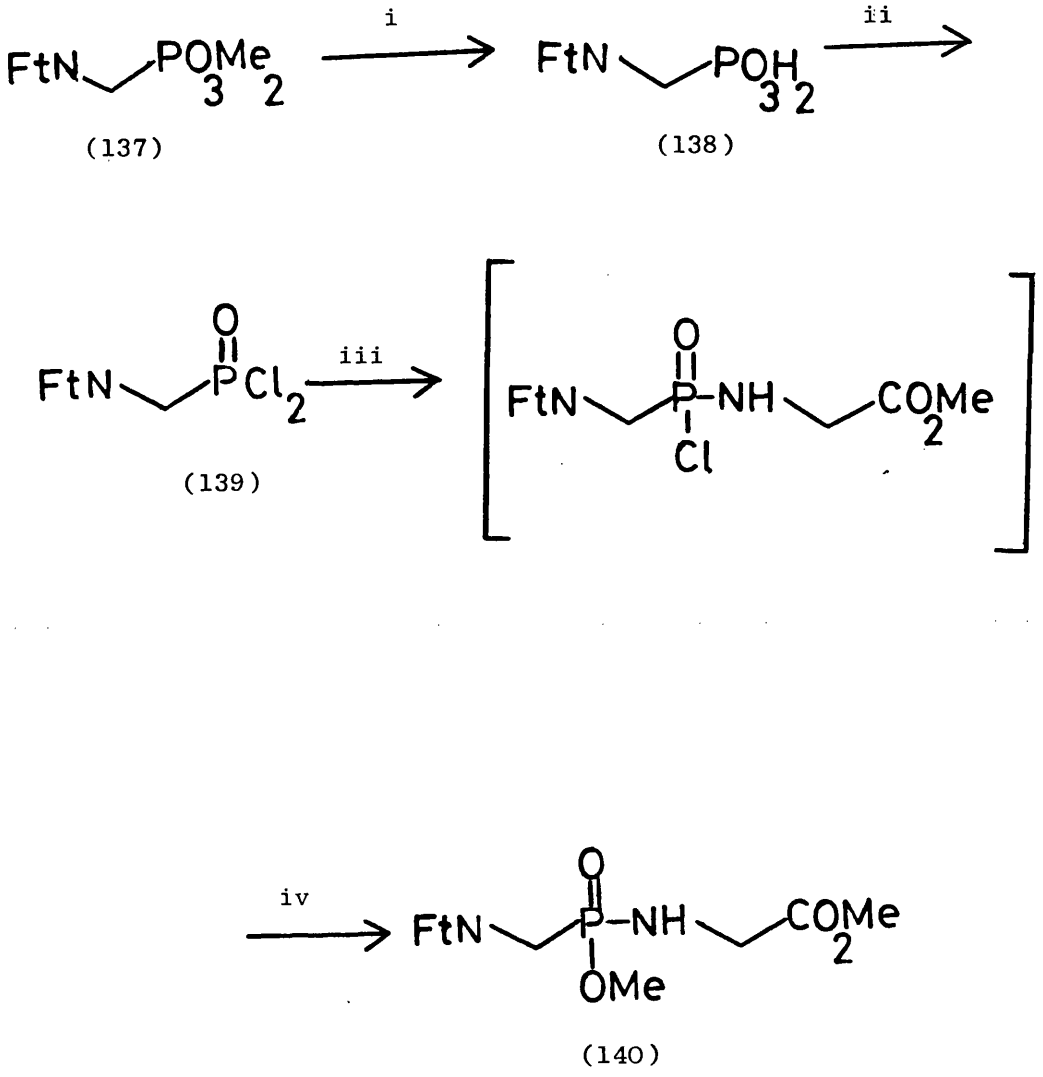
(136) (135)

i, PCl_5; ii, Phenylalanine methyl ester, Et_3N 1 eq. iii, H_2O

(Scheme 38).

The ^{1}H NMR of (136) indicated resonances at δ3.61 (P-OC$\underline{H}_3$, J_{PH} 11Hz) and 3.68 ($CO_2C\underline{H}_3$). The IR spectrum showed stretches at 1780, 1720 (C=O) and 1040 (P=O)cm^{-1}. MS showed m/z 417 (m+1). The structure of (136) was confirmed by CMR and elemental analysis.

We extended the methodology in order to synthesise the phosphonic acid (138) from phalimidomethyl dimethylphosphonate (139) (Scheme 39). Treatment of (137) with excess bromotrimethylsilane followed by hydrolysis of the bis-trimethylsilyl ester produced (138). The structure of (138) was demonstrated by ^{1}H NMR resonances at δ7.8 (aromatic) and 3.7-3.95 (CH_2P, J_{PH} 11Hz). IR spectroscopy indicated 3500-2700 (P-OH), 1710 (C=O) and 1000 (P=O) cm^{-1}. Reaction of (138) with excess thionyl chloride yielded the phosphonochloridate (139). The formation of this key unstable intermediate was indicated by the ^{1}H NMR spectrum in which signals attributed to the methylene protons appeared at lower field δ5.05-4.85 J_{PH} 6Hz relative to the diacid 3.95-3.7 J_{PH} 11Hz and diester 4.18-4.01 J_{PH} 11Hz. Hydrolysis of (139) resulted in the formation of (138) confirming its intermediacy in our synthesis of (140). The phosphonochloridate (139) was then coupled without isolation with glycine methyl ester and the product quenched with methanol to yield (140) (Scheme 39). The structure of (140) was indicated by its ^{1}H NMR spectrum, signals at δ7.8 (aromatic), 3.82 (P-OCH_3, J_{PH} 11Hz) and 3.76 (CO_2Me) were observed. IR spectroscopy showed signals at 1780, 1750, 1710 (C=O), 1220 (OMe) and 1140 (P=O) cm^{-1}.



i, TMSBr 2.5 eq. H_2O/acetone, 1:9; ii, $SOCl_2$, excess.

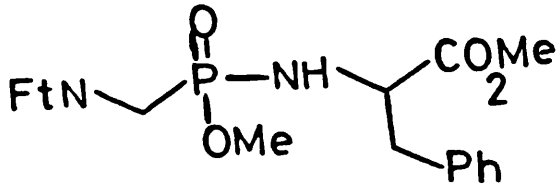
iii, glycine methyl ester, Et_3N 1 eq., iv, MeOH, Et_3N, 1 eq.

(Scheme 39.)

The formation of phosphonamides via phosphonodichloridates such as (139) has not previously been reported. However, the yield of (140) in Scheme 39 was poor (17%).

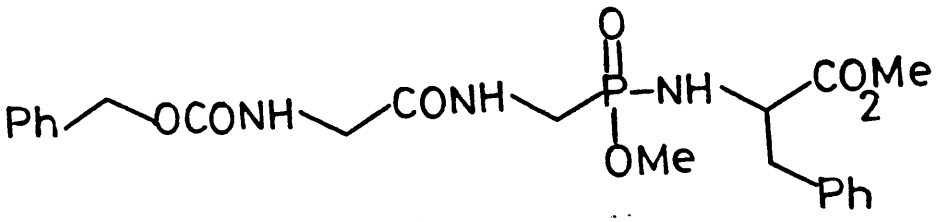
We decided to further extend the above methodology to the synthesis of oligopeptide analogues (142), (143), (144). Our aim was to synthesise an enkephalin analogue containing a phosphonamide moiety.

We prepared tri and tetraphosphonopeptides (142) and (143) respectively from the dipeptide analogue (141), Schemes 40, 41. Key steps in these syntheses involved the removal of the N-phthaloyl functionality with hydrazine and coupling the resulting free amine with an activated ester of an N-protected amino acid or peptide. The structures of (142) and (143) were proven by NMR, IR, MS and elemental analysis.



(141)

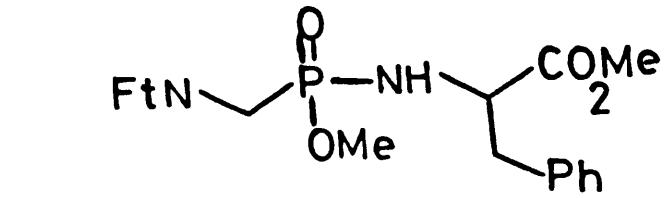
i, ii



(142)

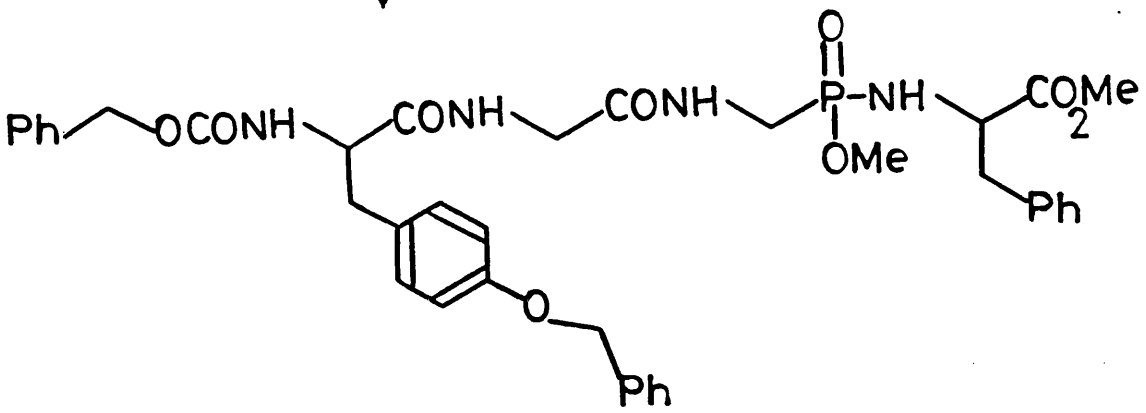
i, NH_2NH_2; ii, ZglyOH, DCC

(Scheme 40).



(141)

i, ii



(143)

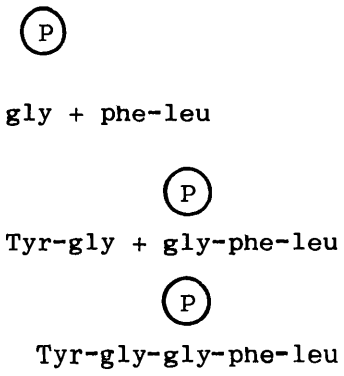
Bzl
i, NH_2NH_2; ii, Z Tyr gly-OH, DCC

(Scheme 41).

A problem encountered in these syntheses was that the dicyclohexylurea (DCU) formed in the coupling reaction was difficult to remove from the product since it streaked into the product upon chromatography. As an alternative we chose diphenylphosphinyl chloride as the reagent for active ester formation. The product from this reagent after coupling is water soluble and thus readily removed.

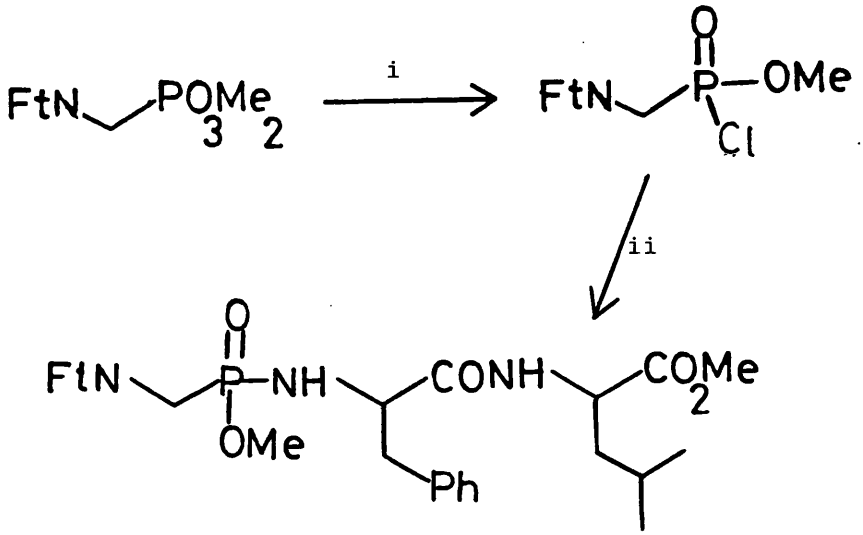
The free amine resulting from the hydrazinolysis of (141) was not isolated. However, its formation was implied by the products formed, e.g. (142) and (143).

Having demonstrated our method of phosphonamide formation and extension of the peptide backbone to be successful we went on to synthesise an endophosphonoenkephalin by a convergent route represented in Scheme 42.



(Scheme 42).

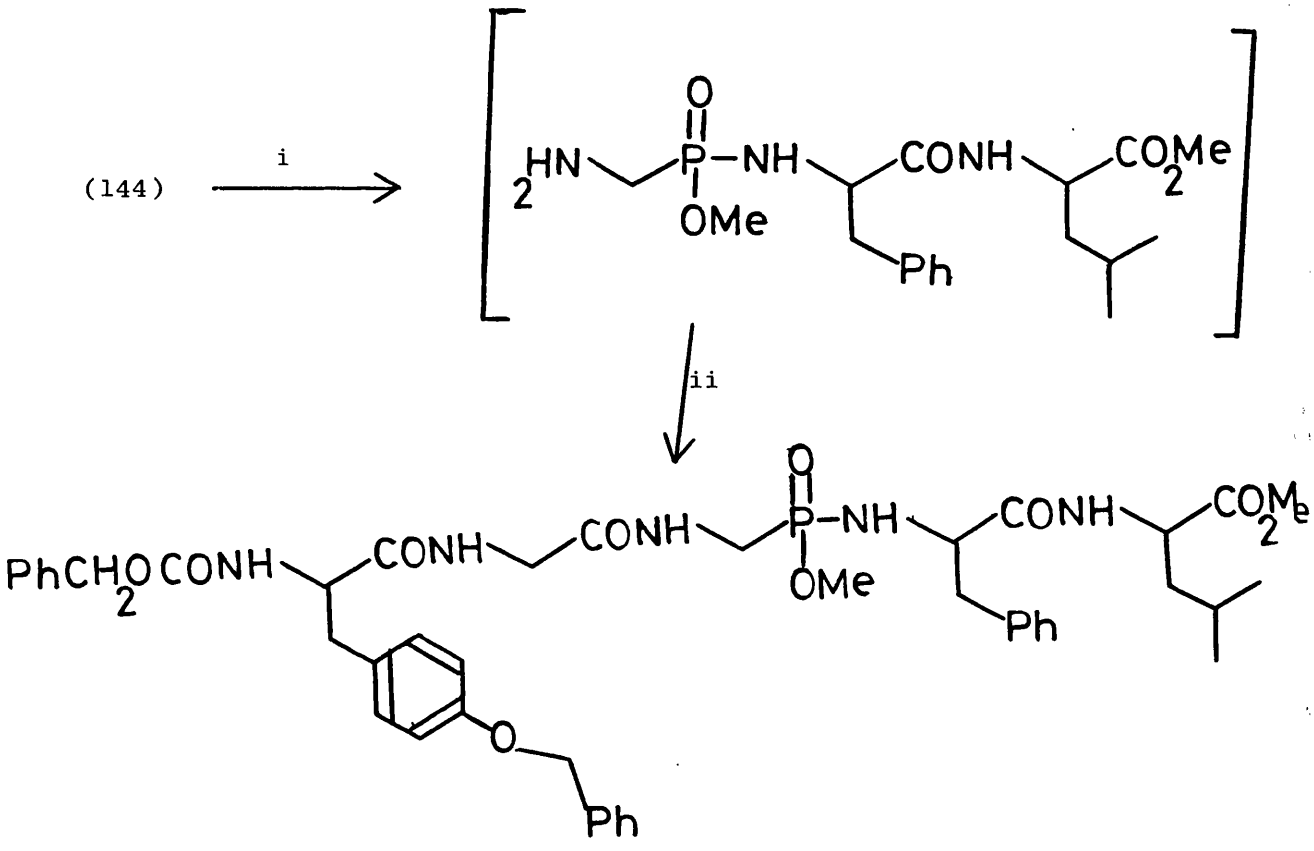
The tripeptide (144) was synthesised as shown in Scheme 43 and its structure demonstrated by ^{1}H-NMR δ7.8-7.3 (aromatic), 3.8 (P-OMe, J_{PH} 11Hz), 3.6 (CO_2CH_3), 0.9 ($(CH_3)_2$). The IR spectrum showed stretches at 1780, 1750, 1710, 1650 (C=O), 1250 (P-OCH_3), 1050 (P=O) cm^{-1}. The structure of (144) was confirmed by MS, m/z 530 (m+1), CMR and elemental analysis.



(144)

i, PCl_5; ii, Phenylalanylleucine methyl ester, Et_3N leq.

(Scheme 43)



(145)

i, NH_2NH_2; ii, ZTyr(Bzl)glyOH + DPPCl + Et_3N

(Scheme 44.)

We then extended the above method to prepare the pentapeptide analogue (145) (Scheme 44). The enkephalin analogue (145) was an important synthetic target. Its structure was elucidated from ^{1}H-NMR which showed resonances at δ 3.5 (P-OCH_3, J_{PH} 11Hz), 3.64 (CO_2CH_3) and 0.88 ($(CH_3)_2$). MS showed m/z 844 (M+1) and amino-acid analysis indicated (gly 1.01, leu 0.92, tyr 0.97, phe 0.91). The yield of (145) from (144) was poor, probably due to hydrazine initiating cleavage of the -P-N system in (144). This was suspected because phenylalanyl-leucine methyl ester was detected by t.l.c. in the reaction mixture. For a practical synthesis of (145) alternative methods of protection and deprotection of (144) need to be established. Furthermore a detailed study of the stability of the -P-N- system in phosphono-peptides should prove useful in order to optimise reaction conditions.

The deprotection of (145) has so far been unsuccessful. The methods of deprotection attempted have included; 1) The use of ammonium formate with 10% Pd/C; 2) Treatment with boron tribromide followed by hydrolysis; 3) Reaction with bromotrimethyl-silane followed by hydrolysis; 4) Reaction with sodium hydroxide.

The attempted deprotection of (145) using bromotrimethylsilane looked the most promising as ^{1}H-NMR indicated the absence of phosphonate ester and benzyoxycarbonyl resonances. However, ^{1}H-NMR also showed that deprotection of the O-benzyl ether was not complete and a signal assigned to the carbomethoxy ester was also present. Reverse phase chromatography indicated that one major product was

present. The product reacted with ninhydrin indicating the presence of a free amino function. The IR spectrum 1740-1650 (C=O) and 1050 (P=O) cm^{-1}. However, no satisfactory mass spectral data was obtained in order to support a structure for this product.

No completely deprotected endophosphonopeptide of this type has ever been isolated. Out attempts at deprotecting (145) and isolation of such a product have proved unsuccessful. The fact that we were unable to isolate a deprotected product leads us to propose that such products might be unstable.

c) Summary

In the above studies we have investigated the coupling methods for formation of phosphonamides. We have also employed our methodology in the synthesis of peptide analogues with two to four units. The synthesis of the target endophosphonoenkephalin has also been accomplished, albeit in fully protected form as conventional deprotection failed to give the free pentapeptide.

Further studies on the stability of the phosphonamide system in these peptide analogues will now be discussed.

3. Kinetic Studies on the hydrolysis of a phosphonamide dipeptide analogue

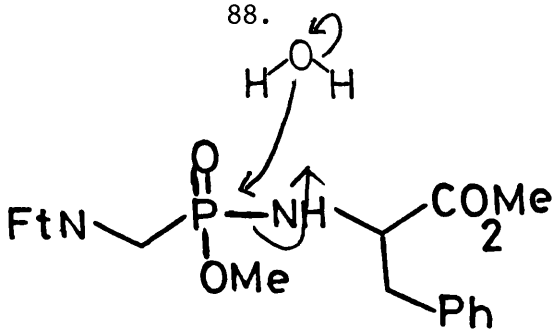
(a) H.P.L.C. Studies on the dipeptide analogue (136)

It has been suggested that phosphonamides are both acid and base labile[178]. However, no studies on the stability of the P-N link of phosphonamide systems in peptides have been reported. As peptides containing phosphonamide bonds may be chemotherapeutically important it is essential that the stability of the phosphonamide system is known over a range of pH values 1 - 12.

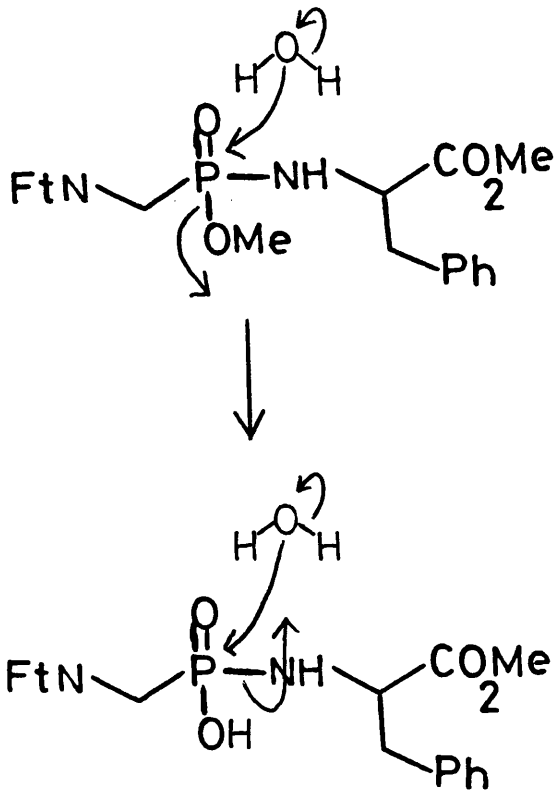
We prepared the dipeptide analogue (136) from phthalimidomethyl dimethylphosphonate as shown in Scheme 38. The product was shown to be spectroscopically pure and homogeneous by h.p.l.c. and t.l.c. By developing h.p.l.c. systems for the detection of (136) and its hydrolysis products the mechanism and rate of hydrolysis of (136) were investigated over a range of pH.

Several mechanisms of hydrolysis involving initial attack at the P-N system were thought to be possible, these included:

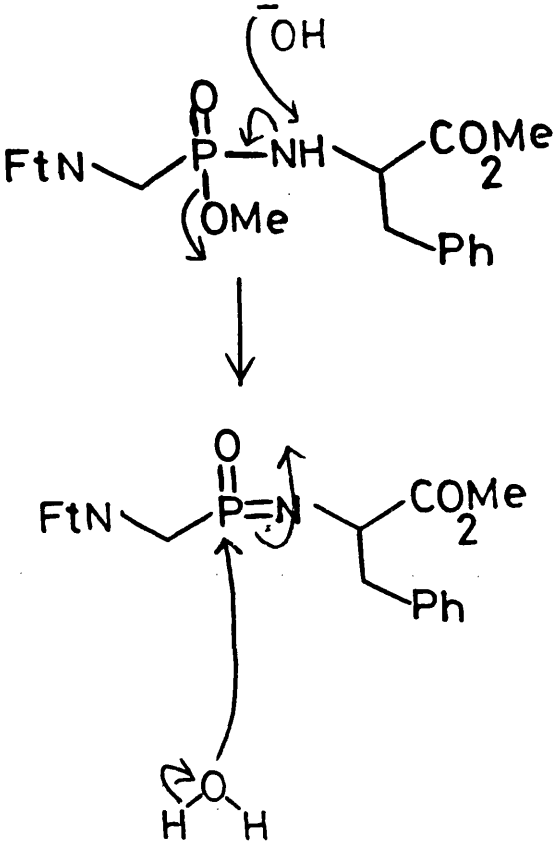
(1) Nucleophilic attack at phosphorus and displacement of phenylalanine methyl ester



(2) Nucleophilic attack at phosphorus and displacement of methoxide, followed by further reaction at the phosphonic system thus formed and displacement of phenylalanine methyl ester.



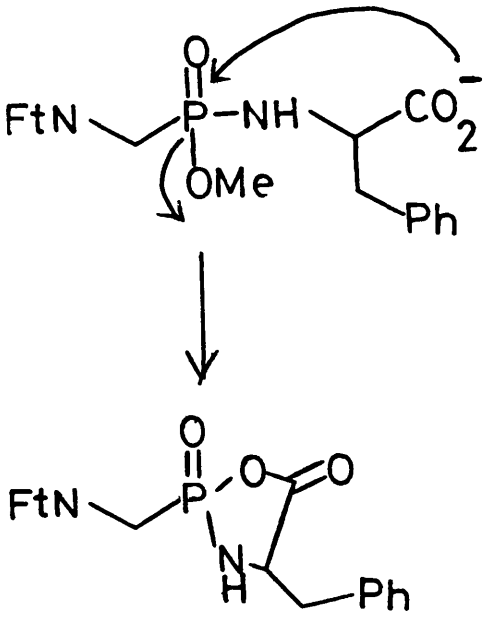
(3) Metaphosphonate formation via an E_1CB pathway with subsequent hydrolysis of the P=N system



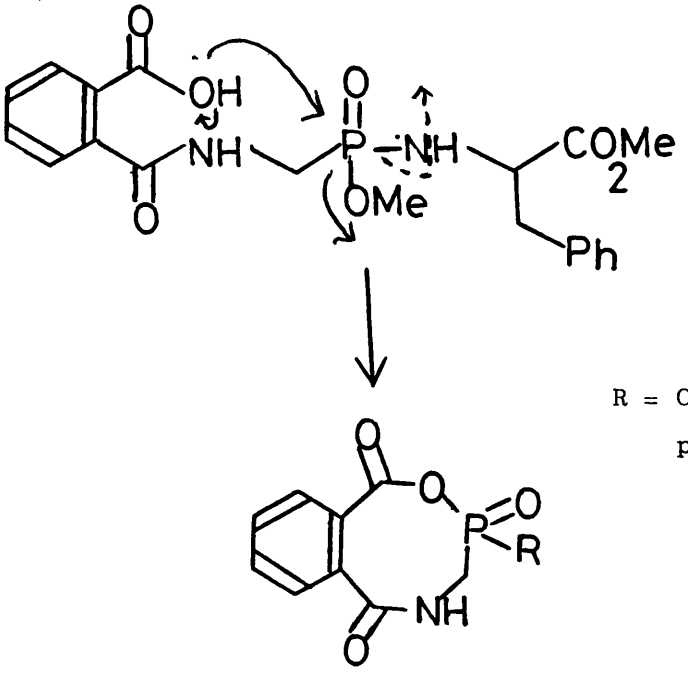
Mechanisms involving hydrolysis of the carbomethoxy ester and phthalimide functions were also considered possible. These included:

(1) Hydrolysis of the carbomethoxy ester and formation of a cyclic anhydride by nucleophilic displacement of methoxide,

followed by hydrolysis of the cyclic anhydride intermediate

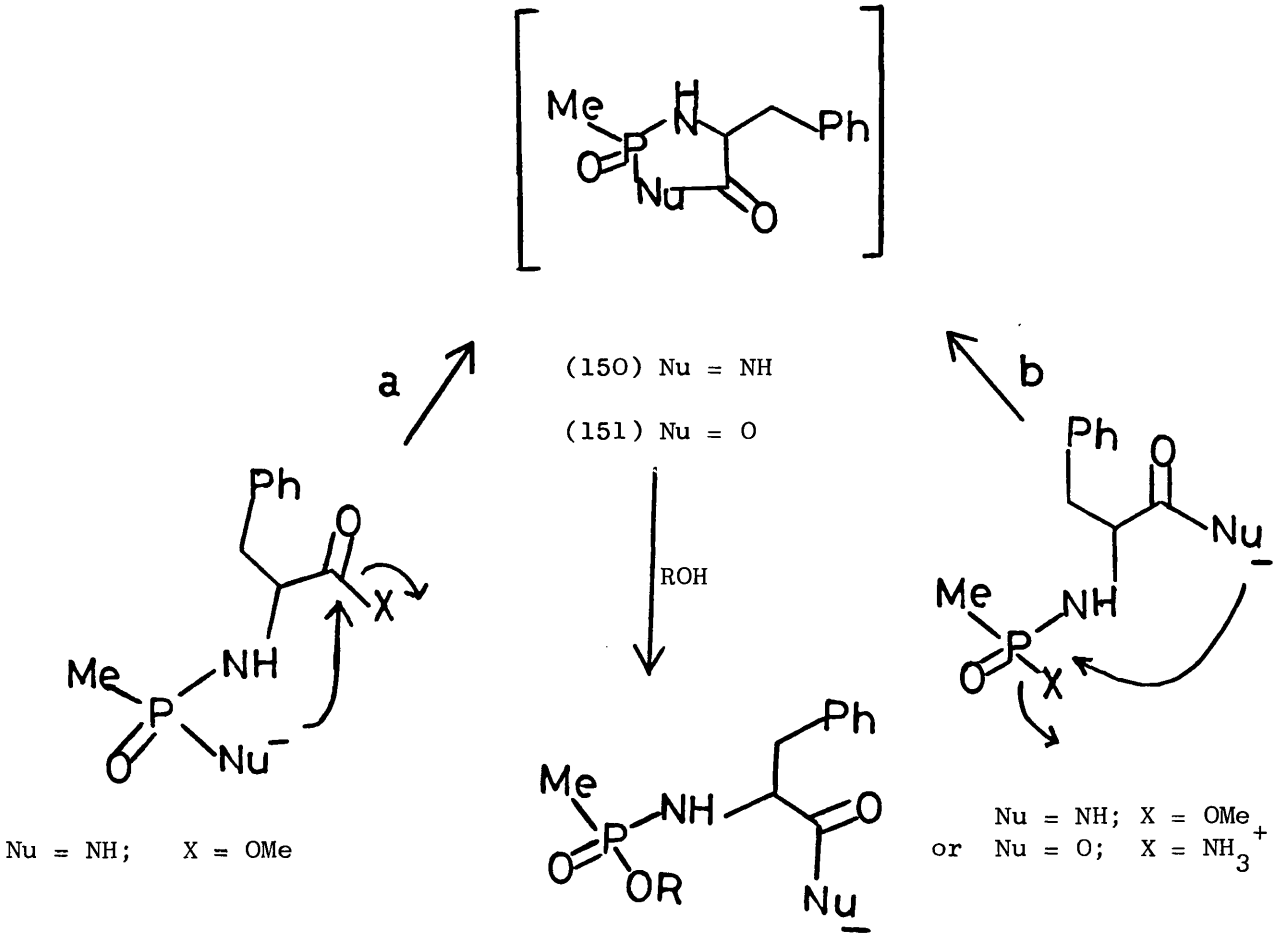


(2) Hydrolysis of the phthaloyl protecting group with subsequent attack at phosphorus and displacement of either methoxide or phenylalanine methyl ester forming a cyclic anhydride. Further hydrolysis of the cyclic anhydride could then occur.



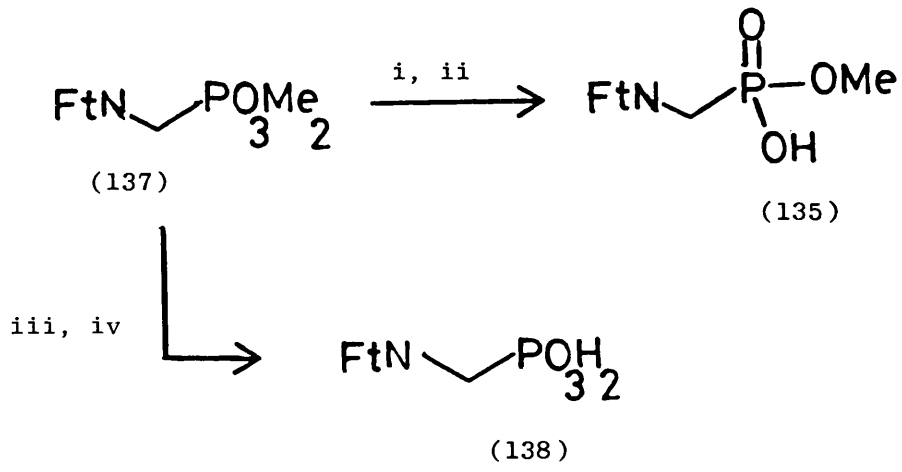
R = OMe or phenylalanyl

Bartlett[176] has recently studied the rearrangement and solvolysis of N-(Amino(methyl)phosphinyl)-L-phenylalanine derivatives. In his proposed mechanistic pathways Bartlett[176] discussed the involvement of transient intermediates (150) and (151) Scheme 44. Such intermediates arose either by cyclisation of a phosphorus-bound nucleophile on the acyl carbon (path a) or by cyclisation of a carbon-bound nucleophile on the phosphorus centre (path b). In hydroxylic solvents, the intermediates (150) and (151) underwent rapid cleavage, exclusively at phosphorus, to give nucleophilic transfer and solvolysis (path a) or catalysed solvolysis, path (b).



In our study the identification of the products of hydrolysis and determination of the order of hydrolysis provided an insight to the mechanisms by which hydrolysis was occurring.

We synthesised the mono- and diphosphonic acids (135) and (138) as shown in Scheme 45. The structures of (135) and (138) were confirmed by NMR, IR, MS, and elemental analysis.



(137)

(135)

(138)

i, PCl_5, ii, H_2O; iii, TMSBr; iv, acetone-water 9:1.

Scheme 45.

Other possible products arising from the hydrolysis of (136) were phenylalanine methyl ester, phenylalanine and phthalic acid. The h.p.l.c. systems that were developed for the detection of the products of hydrolysis using reverse phase chromatography are shown in Table 3. All separations were carried out using Spherisorb 5μ ODS 25mm x 4 m.m. i.d. as the stationary phase.

Having developed chromatographic separations for the hydrolysis products, the hydrolysis of (136) was examined at various pH values (1 - 12). In a typical procedure 20mg of (136) was dissolved in 10 ml of methanol and 100μl of this solution added to 10 ml of buffer solution. A 20μl injection from each buffer solution was taken at different times and the disappearance of the peak due to (136) noted. Detection of the products of hydrolysis was carried out using 100μl injections from the hydrolysates in the chromatographic systems developed, see Table 4.

Table 4.

Compound	Mobile phase	flow rate ml/in	Retention time (min)
(136)	methanol, 65 water, 25 0.1 M Na_2HPO_4, 5 0.1 M NaH_2PO_4, 5 pH 7	1.5	7.21
(138)	citrate buffer, 75 pH 3.2 methanol, 25	1.5	4.01
(135)	citrate buffer, 75 methanol, 25 pH 3.2	1.5	4.96
phe OMe	phosphate buffer, 30 methanol, 70 pH 8.2	1.0	5.65
phe	phosphate buffer, 50 methanol, 50 pH 8.2	1.5	4.97
phthallic acid	phosphate buffer 50 methanol 50 pH 8.2	1.5	4.18

Table 5 shows the pH range investigated during our stability studies.

Table 5.

pH	Solutions used to obtain correct pH
1	0.1 M HCl
1.5	HCl 0.2 M/KCl 0.2 M 1:1
2.6	0.1 M $NaClO_4$/$^{M}/_{100}$ $HClO_4$ [a]
3.1	citrate buffer
4	0.1 M NaOAc/0.1 M HOAc [b]
7	NaH_2PO_4 0.1 M/Na_2HPO_4 1:1
8.5	citric acid/NaOH [c]
9.5	citric acid/NaOH [c]
10	citric acid/NaOH [c]

a pH adjusted by addition of $^{M}/_{100}$ $HClO_4$ to 0.1 M $HClO_4$

b pH adjusted by addition of 0.1 M HOAc to 0.1 M NaOAc

c pH adjusted by addition of 2M NaOH to 2M citric acid

Our h.p.l.c. studies showed conclusively that (136) was stable between pH 1-7 at room temperature, i.e. no decrease in concentration of (136) was observed in the solutions between pH 1-7 after several hours. Hydrolysis of (136) occurred at 60°C at pH 1 with pseudo-first order kinetics. A logarithmic plot of concentration of (136) against time gave a straight line. Both phenylalanine methyl ester and phthalimidomethyl-O-methyl phosphonic acid (135) were detected in the hydrolysis mixture.

Under basic conditions hydrolysis of (136) was more facile. Below pH 10 pseudo-first order kinetics were observed for the hydrolysis of (136). A logarithmic plot of concentration of (136) against time gave a straight line. Again both phenylalanine methyl ester and phthalimidomethyl-O-methyl phosphonic acid (135) were detected in the hydrolysis mixtures.

The chromatographic systems used for product detection were carefully chosen in order that other possible hydrolysis products could be detected. However, no other products were detected.

The rate constants, half life of hydrolysis of (136) and correlation coefficients for the pseudo-first order hydrolysis of (136) are shown in Table 6.

Table 6.

pH	rate constant (s^{-1})	Half life (s)	correlation coefficient
1 60°C	1.9×10^{-4}	3.56×10^{3}	0.999
9.5	4.48×10^{-5}	1.51×10^{4}	0.996
10.0	4.5×10^{-4}	1.51×10^{3}	0.995

The computer "print-outs" for the plots of log concentration against time for the above pH ranges are shown below.

NO	T(MIN)	OBS A	% REACT	CALC A	% REACT	RESID
1	1.100	9408.000	2.472	9531.914	1.220	-1.2391E+02
2	10.300	8582.000	10.822	8578.825	10.854	3.1752E+00
3	19.650	7867.000	18.050	7705.392	19.684	1.6161E+02
4	30.100	7086.000	25.945	6831.172	28.521	2.5483E+02
5	45.530	5431.000	42.676	5713.096	39.824	-2.8210E+02
6	61.830	4591.000	51.167	4723.417	49.829	-1.3242E+02
7	86.880	3614.000	61.044	3513.471	62.060	1.0053E+02
8	104.880	2849.000	68.777	2830.713	68.962	1.8287E+01

K = 1.1155E-02(M-1) LOG(K) = -1.9525 HALF LIFE = 6.2137E+01 MIN
K = 1.8592E-04(S-1) LOG(K) = -3.7307 HALF LIFE = 3.7282E+03 SEC
P=A+B*EXP(-K*T) A=P(INFINITY)= -239.622 P(0)= 9652.556
FRACTION CHANGE IN K IN CYCLE 19 = 1.3203E-09
OVERRELAXATION FACTOR IN CYCLE 19 = 2.00
ST DEV FOR K = 6.1291E-04 (5.494 %) CORR COEFF = 0.99956
ST DEV FOR A = 1.4628E+02 (1.479 %) 1-R*R = 8.6577E-0

NO	T(MIN)	CONC	LN(X)	LN(X)EST-LN(X)
1	0.93	84935.000	0.0000	0.0128
2	7.43	80533.000	0.0532	-0.0226
3	15.08	79905.000	0.0610	-0.0094
4	31.10	79125.000	0.0709	0.0249
5	166.06	52644.000	0.4783	-0.0114
6	250.48	42453.000	0.6935	0.0056
*	70.83	61090.000	0.3295	-0.1245
	INFINITY	0.000		

K = 4.5840E-05(S-1) LOG(K) = -4.339 HALF LIFE = 1.5121E+04 SEC
K = 2.7504E-03(M-1) LOG(K) = -2.561 HALF LIFE = 2.5202E+02 MIN

CORR COEFF = 0.996281 ST ERROR = 1.4003E-06 S-1 (3.055 %)

NO	T(MIN)	CONC	LN(X)	LN(X)EST-LN(X)
*	0.67	70020.000	0.0000	0.2667
2	7.42	49011.000	0.3567	0.0880
3	14.22	38919.000	0.5873	0.0366
4	20.98	29235.000	0.8734	-0.0713
5	28.96	25822.000	0.9976	0.0149
6	35.96	21107.000	1.1992	-0.0022
7	43.23	15920.000	1.4812	-0.0926
8	79.55	6859.000	2.3232	0.0227
9	102.35	3918.000	2.8832	0.0637
10	72.75	7555.000	2.2266	-0.0599
	INFINITY	0.000		

K = 4.3931E-04(S-1) LOG(K) = -3.357 HALF LIFE = 1.5778E+03 SEC
K = 2.6358E-02(M-1) LOG(K) = -1.579 HALF LIFE = 2.6297E+01 MIN

CORR COEFF = 0.994776 ST ERROR = 1.2032E-05 S-1 (2.739 %)

Above pH 10 hydrolysis of (136) occurred rapidly and phthalic acid was detected in the hydrolysis mixtures. Thus the phthalimido moiety of (136) was being hydrolysed at elevated pH. From our studies we knew that (136) hydrolysed at pH 10 with a half-life of 1.51×10^3 sec. ($\approx$ 25 mins). Hence higher pH must be minimised or avoided completely in any manipulations with phosphonamides such as (136).

The product analysis of the hydrosylates between pH 1 - 10 suggest that the mechanism of hydrolysis is attack at phosphorus with displacement of phenylalanine methyl ester.

(b) Summary

Our studies showed that (136) hydrolyses *via* pseudo-first order kinetics between pH 1 - 10. The mechanism of hydrolysis was implied from the hydrolysis products observed.

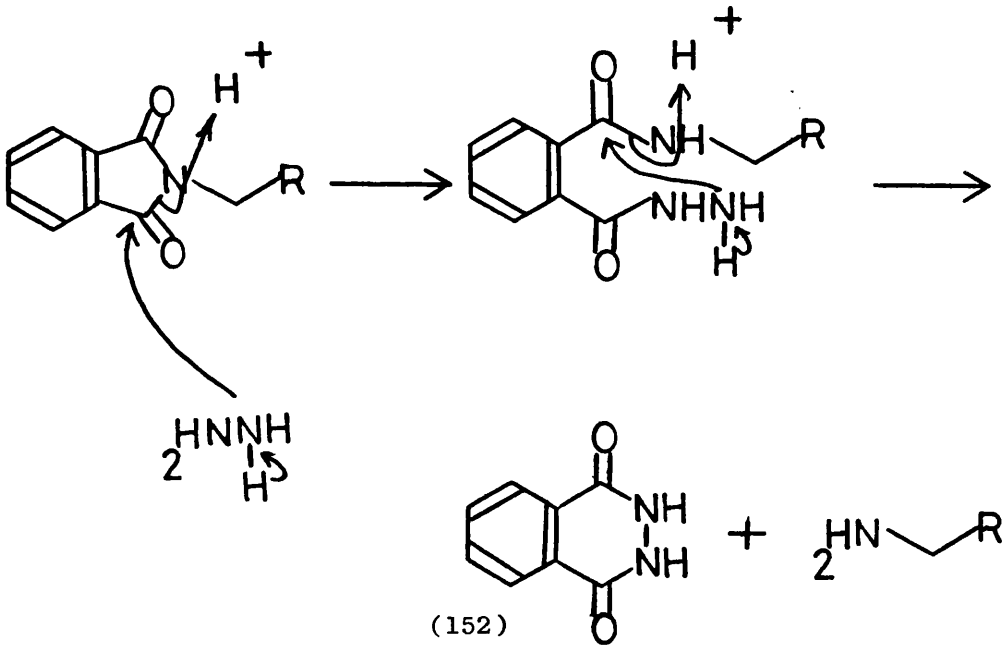
Having investigated the stability of a phosphonamide system over a range of pH we now have a far better guide of the pH limits under which experiments may be conducted with peptide analogues containing a phosphonamide system .

As our present method of removal of phthalimide involves hydrazine, which is both nucleophilic and basic, it seems reasonable that the conditions of the reaction result in some cleavage of the phosphonamide system. Our studies therefore led us to investigate other possible N-protection that would be labile under less basic conditions.

4. Studies on N-phthaloyl deprotection and the synthesis of novel N-protected dimethyl phosphonates

a) Removal of phthalimide by hydrazinolysis

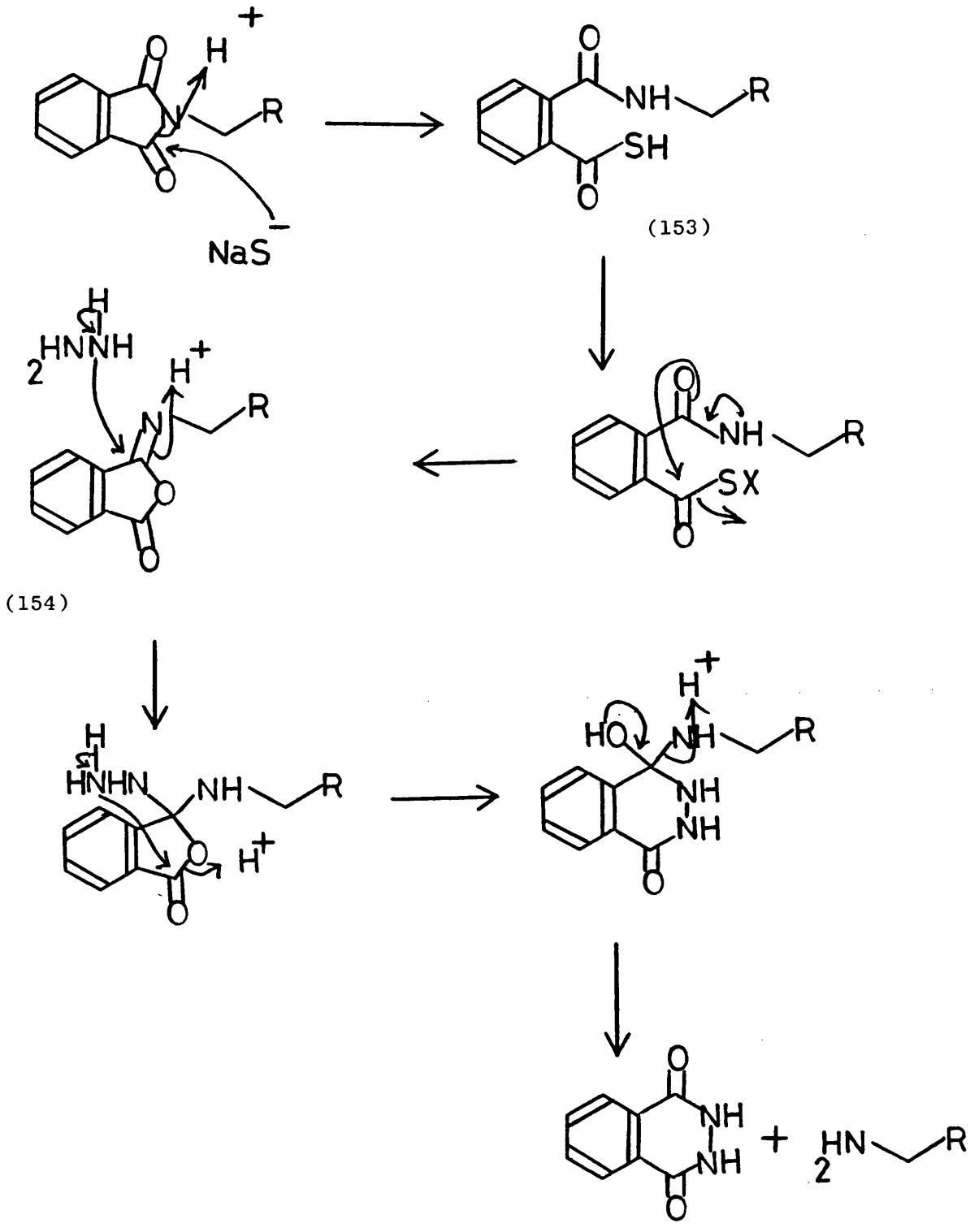
The synthesis of phosphonamides utilised in our study involved coupling of N-phthaloyl phosphonochloridates with amino acid or peptide esters. Although phthalimide is an excellent protecting group its removal has proved troublesome in our hands. Until recently the most common methods of phthalimide removal involved hydrazinolysis, i.e. strongly basic conditions, Scheme 46. We have successfully used hydrazine to remove phthalimide from phosphonopeptides, however, the yield was low due to P-N bond cleavage of the phosphonamide. Also the by-product of hydrazinolysis the phthalhydrazide (152) proved difficult to separate from the desired product.



(152)

(Scheme 46).

The use of methylhydrazine was reported [177], an advantage being that the N-methyl phthalhydrazide by-product was more easily separable from the product. However, in our case yields were not improved using methylhydrazine as the basicity was still thought high enough for P-N bond cleavage.

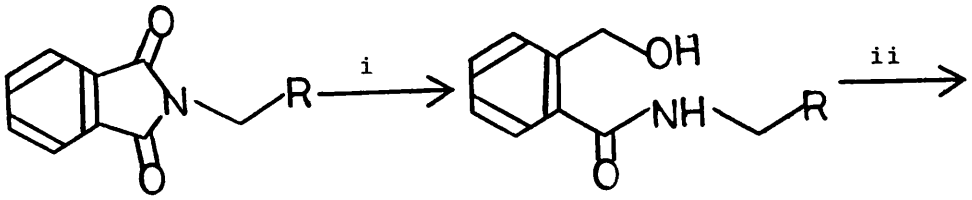


(Scheme 47.)

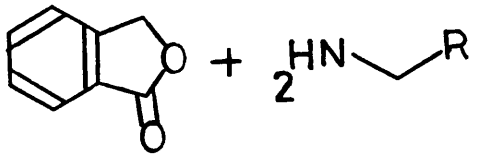
A milder, less basic system using sodium sulphide has been described[178]. In this case the acidic intermediate (153) was activated using mixed anhydride methods[178], yielding an iso-imide (154). Hydrazinolysis of the iso-imide formed was reported[178] to be more facile than phthalimide, Scheme 47. When applied to our phosphonopeptides no improvement in yield after deprotection of phthalimide was observed. From our observations above, and stability studies on (136) over a range of pH it was apparent that if yields of deprotected product were to be improved, basic conditions would have to be minimised or avoided altogether.

b) Removal of phthalimide using sodium borohydride

Recently Ganem[179] described a novel method of phthalimide cleavage. The phthalimide group was treated with sodium borohydride in propan-2-ol affording an α-hydroxymethyl benzamide analogue (155), which upon treatment with acetic acid produced the deprotected product (156) and an easily separable by-product (see Scheme 48.)



(155)



(156)

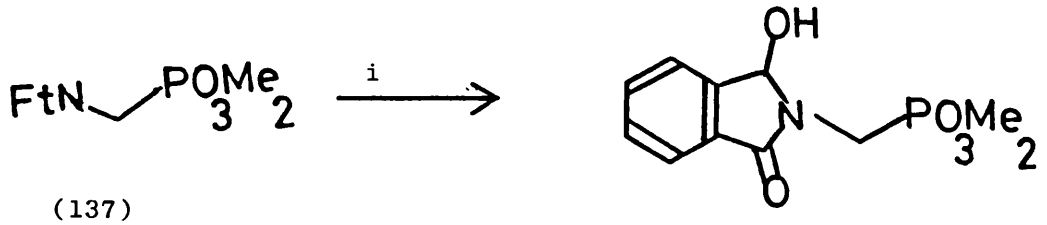
i, $NaBH_4$, propan-2-ol; ii, AcOH

(Scheme 48.)

When applied to our systems, the use of excess sodium borohydride was unsuccessful, with P-N bond cleavage observed, and phenylalanyl-leucine methyl ester detected by t.l.c. Treatment of (137) and (144) with 1 equivalent of sodium borohydride afforded the α-amidoalcohols (157) and (158) (Scheme 49). Compound (157) exhibited characteristic stretching frequencies in the IR spectrum at 3350(OH), 1700(C=O), 1050(P=O) cm^{-1}. The mass spectrum showed a molecular ion m/z 271 and the ^{1}H-NMR and CMR showed the presence of -CH-OH with shifts of δ 6.01 and δ 167 for ^{1}H and ^{13}C respectively.

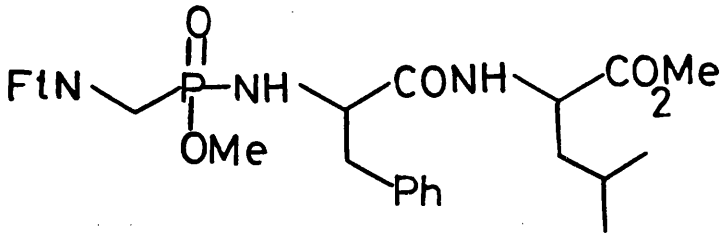
Similarly compound (158) showed in the IR spectrum, 3350 (OH), 1740 (CO_2Me), 1680 (CONH) and 1050 (P=O) cm^{-1}. ^{1}H-NMR indicated resonances at δ6.01 and 5.90 (CHOH), 3.62 (CO_2Me), 0.85 ($(CH_3)_2$). These resonances were also observed in the CMR spectrum at δ82.5 (CHOH), 52.17 ($O\underline{C}H_3$), 22.8 and 21.7 ($(CH_3)_2$). FAB MS showed m/z 532 (M+1) and 530 (M-1).

The α-amidoalcohols (157) and (158) were stable when heated with acetic acid. As excess borohydride is required to form the α-hydroxymethyl benzamide analogues such as (155) and our systems are not stable to excess borohydride this methodology was not further investigated.

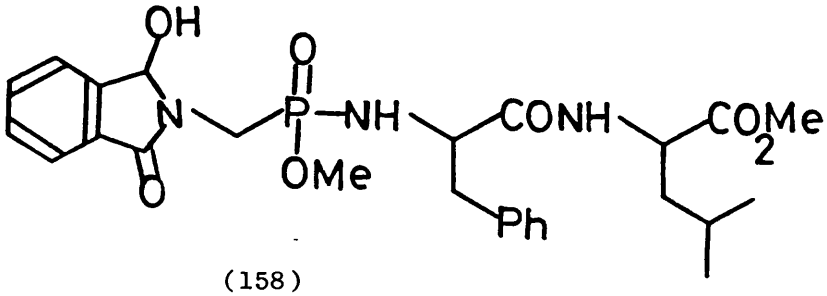


(137)

(157)



(144)



(158)

i, $NaBH_4$ 1 eq, propan-2-ol.

(Scheme 49).

c) Summary

We have employed hydrazine in the deprotection of N-phthaloyl phosphonopeptides. The basic conditions resulting from hydrazinolysis are such that low yields of desired products are observed due to phosphonamide P-N bond cleavage. It would therefore be advantageous to investigate other forms of N-protection for our systems in order to improve yields of deprotection and hence coupling.

5. Synthesis of some novel N-protected aminoalkylphosphonate esters.

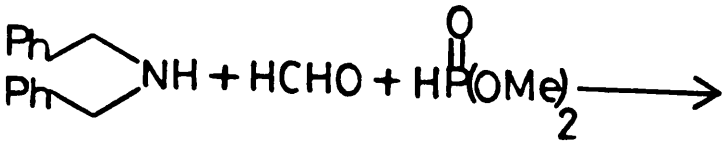
Introduction

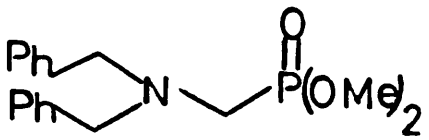
This section is concerned with our approaches to the synthesis of some new N-protected aminoalkylphosphonate esters with the aim of developing a protecting group that would:

1) survive the conditions of phosphonamide formation, and
2) could be easily removed after coupling was effected.

a) The N,N-dibenzyl protecting group

Recently the N,N-dibenzyl phosphonate (159) was reported[180] and synthesised as shown in Scheme 50.

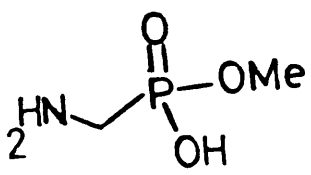




(159)

(Scheme 50).

The removal of N,N-dibenzyl protection has been effected[181] using palladium black and 4.4% formic acid in methanol as a hydrogen donor. We investigated this approach for the removal of the N,N-dibenzyl groups in (159). In our studies we observed that reaction of (159) with palladium black and 4.4% formic acid in methanol not only cleaved the N,N-dibenzyl protection but also removed one of the phosphonate methyl esters, giving (160) as the product. The ^{1}H-NMR of (160) showed only two doublets integrating in a ratio 3:2 δ 3.1 (CH_2P, J_{PH} 12Hz), 3.6 ($P-OCH_3$, J_{PH} 10Hz). The CMR of (160) also indicated two doublets δ 59.33 and 52.95 ($P-O\underline{C}H_3$, J_{CP} 6.1Hz), 38.3 and 31.9 ($\underline{C}H_2P$, J_{CP} 144Hz). FAB MS showed m/z 126 (M+1) and 124 (M-1).



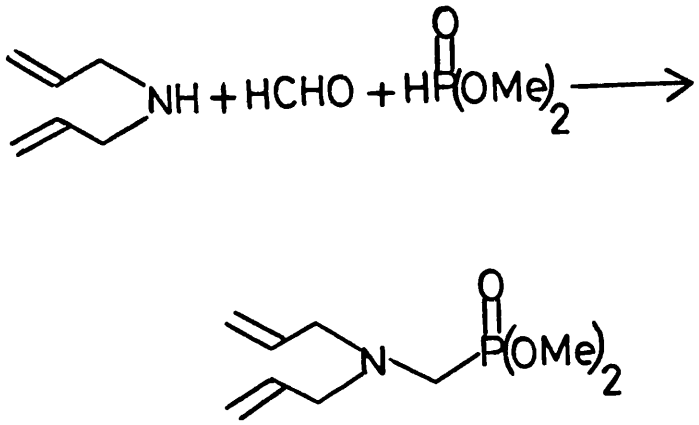
(160)

When (159) was treated with phosphorus pentachloride followed by attempted phosphonamide formation with an amino acid or peptide ester unreacted starting material was recovered. Apparently (159) was not reacting with phosphorus pentachloride to give the required phosphonochloridate. This lack of the required reactivity may possibly be due to the lone pair on the tertiary amine complexing with the phosphorus pentachloride, thereby preventing the required reaction from occurring.

b) The N,N-diallyl protecting group

The N,N-diallyl protecting group has also been employed[182] and has been found to be easily removed using tris(triphenylphosphine)rhodium(I) chloride (Wilkinson's catalyst), followed by acid treatment[183].

We synthesised the novel N,N-diallyl phosphonate (161) in a similar manner to the N,N-dibenzyl analogue (159) Scheme 51. ^{1}H-NMR showed resonances at δ 5.65-6.1 and 5.1-5.35 (CH_2=CH-), 3.75 (P-OCH_3, J_{PH} 11Hz). The IR spectrum indicated stretches at 1450(C=C) 1250 (P-OCH_3), 1050 (P=O) cm^{-1}. Mass spectrometry showed a molecular ion at m/z 219 (M+). The structure of (161) was demonstrated by CMR and its elemental composition was confirmed by high resolution mass spectrometry.



(161)

(Scheme 51).

Wilkinson's catalyst is known[183] to isomerise N-allyl groups to the corresponding enamines. Acidic treatment of which results in the formation of free amines[183]. In our case, after treatment of (161) with Wilkinson's catalyst, followed by acid treatment, it appeared from NMR evidence that only one of the N-allyl groups had been cleaved. The integration over the N-allyl region relative to the phosphorus ester region indicated only one N-allyl group was present.

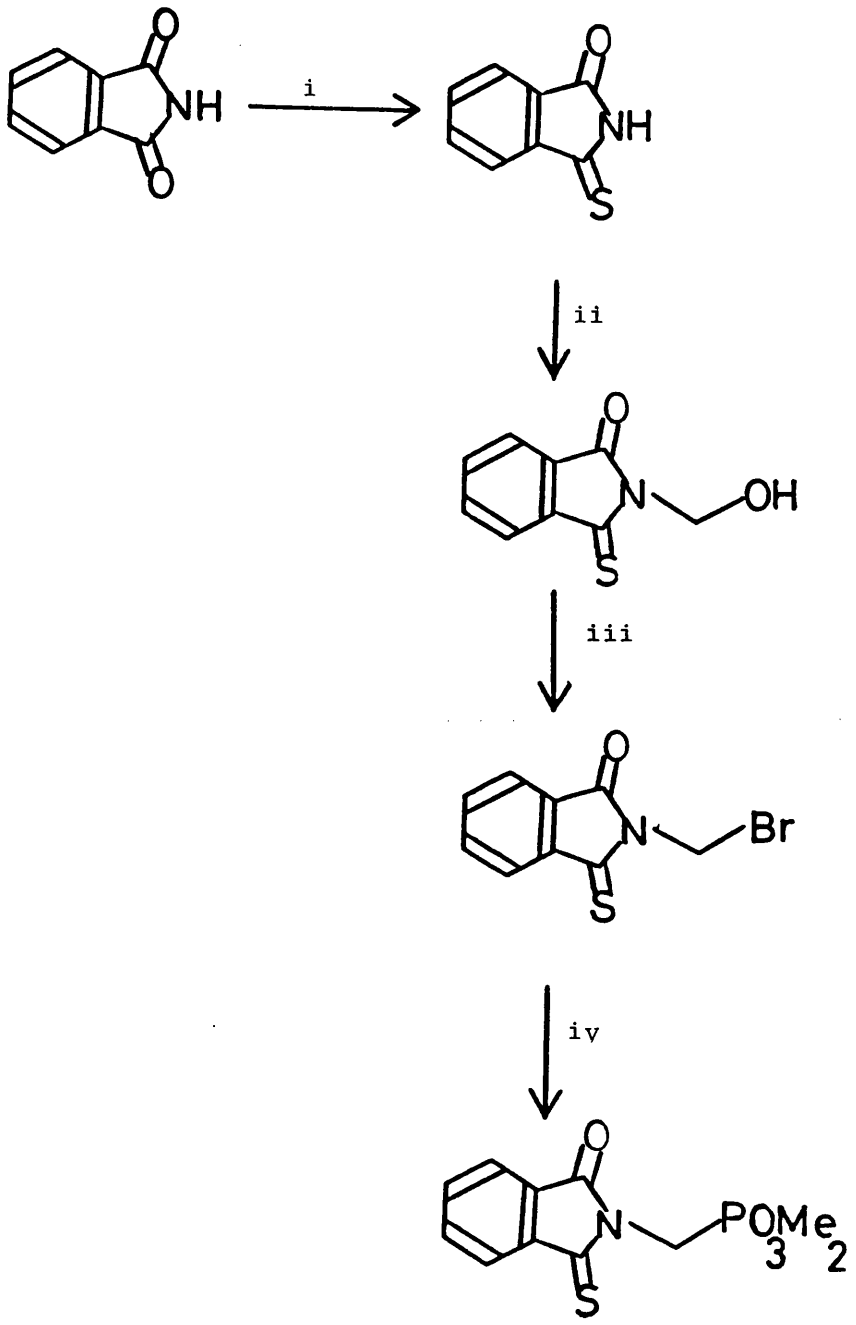
Attempted reaction of (161) with phosphorus pentachloride and subsequent phosphonamide formation with peptide esters was unsuccessful, only unchanged N,N-diallyl phosphonate (161) being recovered. Again the possibility of the lone pair of the tertiary amine complexing with the phosphorus pentachloride cannot be excluded, although the exact reason for the failure of this reaction sequence is not clear.

c) Mono- and dithiophthalimide protecting groups

Mono- and dithiophthalimide have been prepared by Lawesson et al[184]. The use of mono- and dithiophthalimide as protecting groups has not been reported.

We synthesised the novel dimethyl phosphonates (162) and (163) as shown in Schemes 52 and 53 respectively. Compound (162) showed ^{1}H NMR resonances at δ4.35-4.6 (CH_2P, J_{PH} 12Hz), 3.55-3.75 (P-OCH_3, J_{PH} 11Hz) and CMR resonances at δ195.5 (C=S), 168.6 (C=O), 53.0 and 53.2 (P-O$\underline{C}H_3$, J_{CP} 4.87Hz), 39.3 and 32.4 ($\underline{C}H_2P$, J_{CP} 157 Hz). The IR spectrum of (162) showed characteristic stretches 1680 (C=O), 1350 (C=S), 1050 (P=O) cm^{-1}. High resolution mass spectrometry on (162) confirmed its elemental composition.

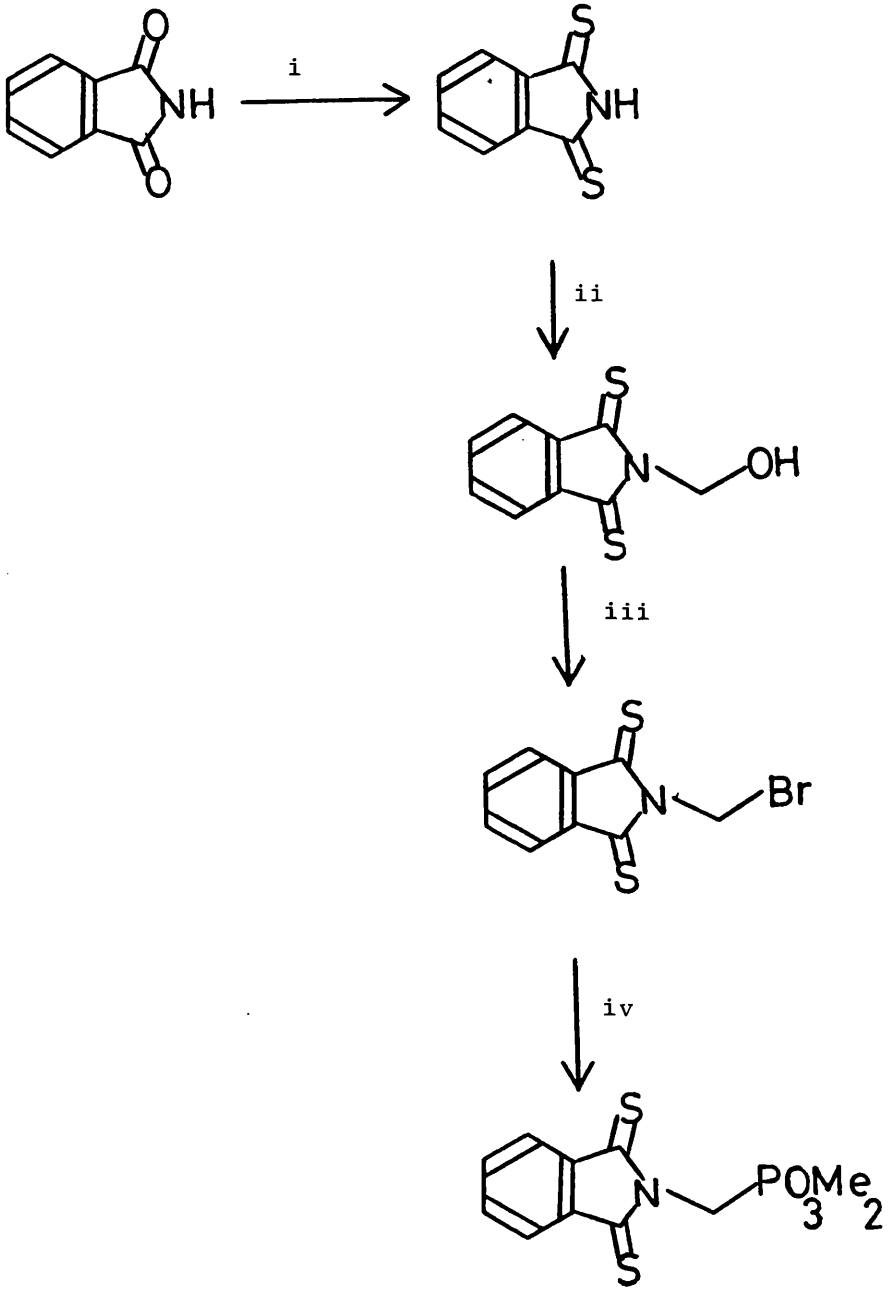
The structure of compound (163) was demonstrated from its ^{1}H NMR spectrum which indicated signals at δ 4.9-5.04 (CH_2P, J_{PH} 12Hz), 3.7-3.85 (P-OCH_3, J_{PH} 11Hz), and CMR spectrum which showed resonances at 195.9 ($\underline{C}$=S), 53.1 and 52.9 (P-O$\underline{C}H_3$, J_{CP} 6.94 Hz), 42.6 and 35.7 ($\underline{C}H_2P$), J_{CP} 146.5 Hz). In the IR spectrum of (163) stretches were observed at 1350 (C=S), 1150 (P-OCH_3), 1050 (P=O)cm^{-1}. High resolution mass spectrometry on (163) confirmed its elemental composition. All other compounds synthesised from either mono- or dithiophthalimide were novel and their structures confirmed by their NMR, IR, MS, UV spectra and either high resolution mass spectrometry or elemental analysis as in the cases of (162) and (163).



(162)

i, Lawesson's reagent; ii, HCHO (aq); iii, PBr_3; iv, $P(OMe)_3$

(Scheme 52).



(163)

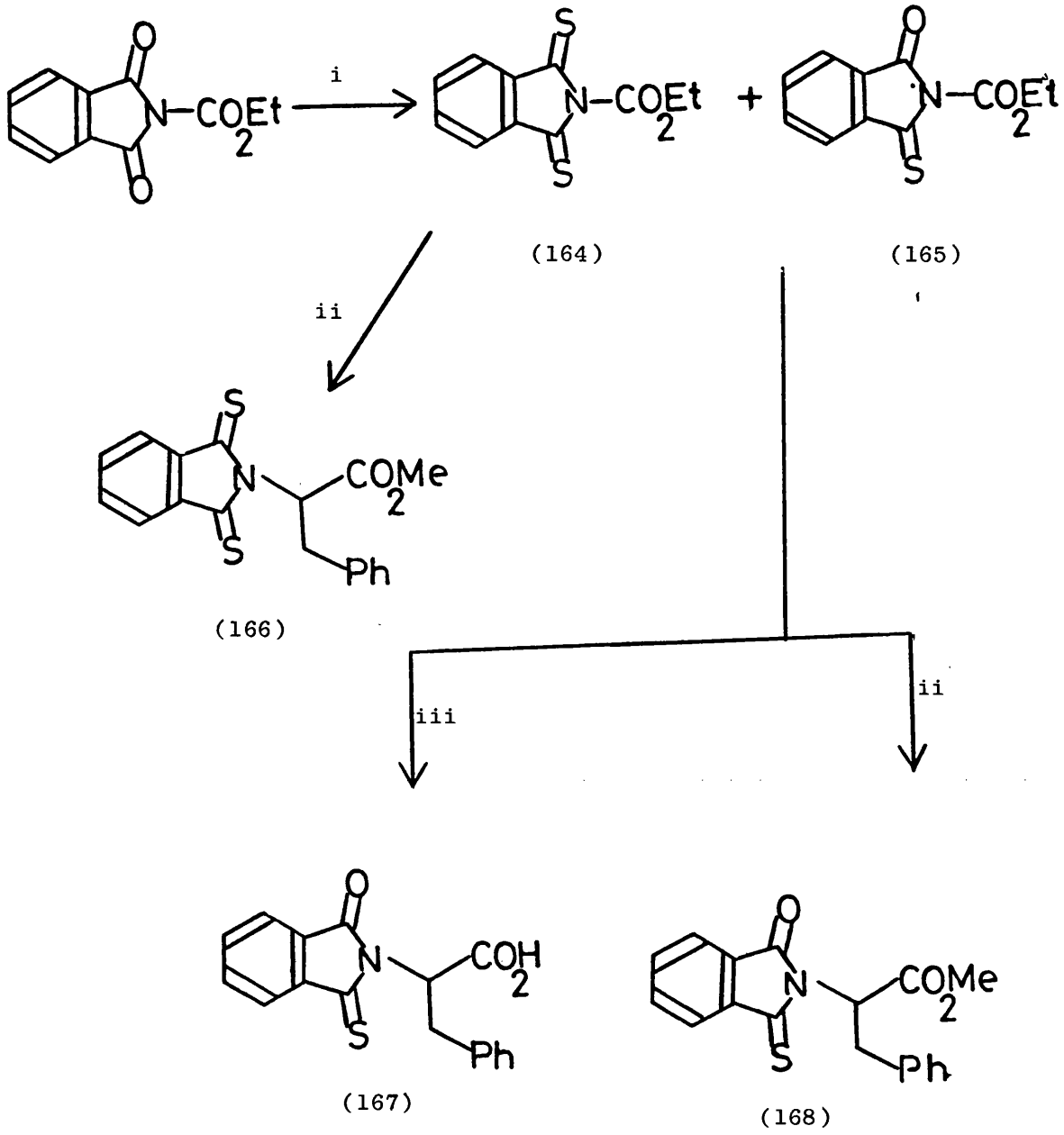
i, Lawesson's reagent; ii, HCHO (aq.); iii, PBr_3; iv, $P(OMe)_3$

(Scheme 53).

An interesting feature of compounds containing mono- or dithiophthalimide groups was their colour. Compounds with dithiophthalimide protection were dark red to black crystalline materials and compounds with monothiophthalimide protection were light red crystalline solids. Both classes of compounds were shown to have characteristic UV spectra. For example compound (162) showed λ_{max} (EtOH) 327nm (ε 7,143) and compound (163) indicated λ_{max} (EtOH) 355nm (ε 19,095).

However, attempted reaction of (162) and (163) with phosphorus pentachloride followed by phosphonamide formation with peptide esters was unsuccessful. Only starting materials were recovered. The reason for this was unclear.

We have, however, demonstrated that mono- and dithiophthalimide protected amino acids can be synthesised by thionating N-carbethoxy phthalimide followed by treatment with the required amino-acid or amino-acid ester (Scheme 54).



i, Lawessons reagent; ii, phenylalanine methyl ester hydrochloride, Et_3N; iii, Phenylalanine, Na_2CO_3.

(Scheme 54).

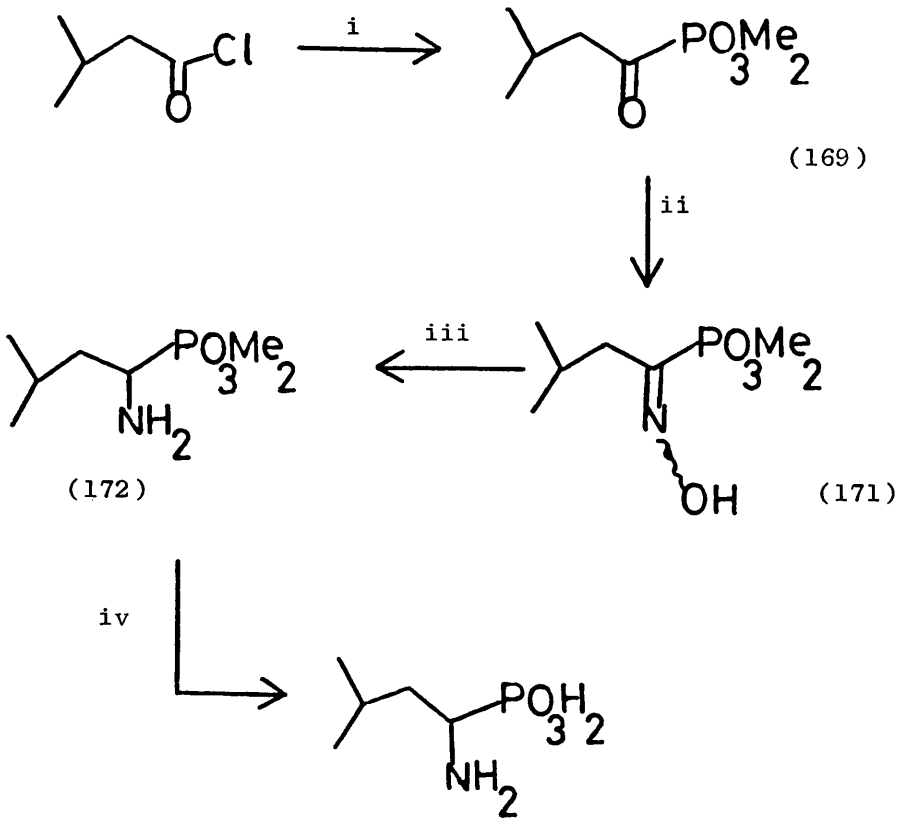
All compounds in Scheme 54 were characterised by NMR, IR, UV, MS or high resolution mass spectrometry.

d) Summary

Methods of N-protection have been examined and some new N-protected phosphonate esters were synthesised. The N-protected phosphonate esters synthesised did not form the required phosphonochloridates using our methodology. The applications of mono- and dithiophthalimide N-protection whilst not successful for our synthetic problem could still find a more general application in amine functional group protection and may eventually provide a viable alternative to some of the more conventional systems.

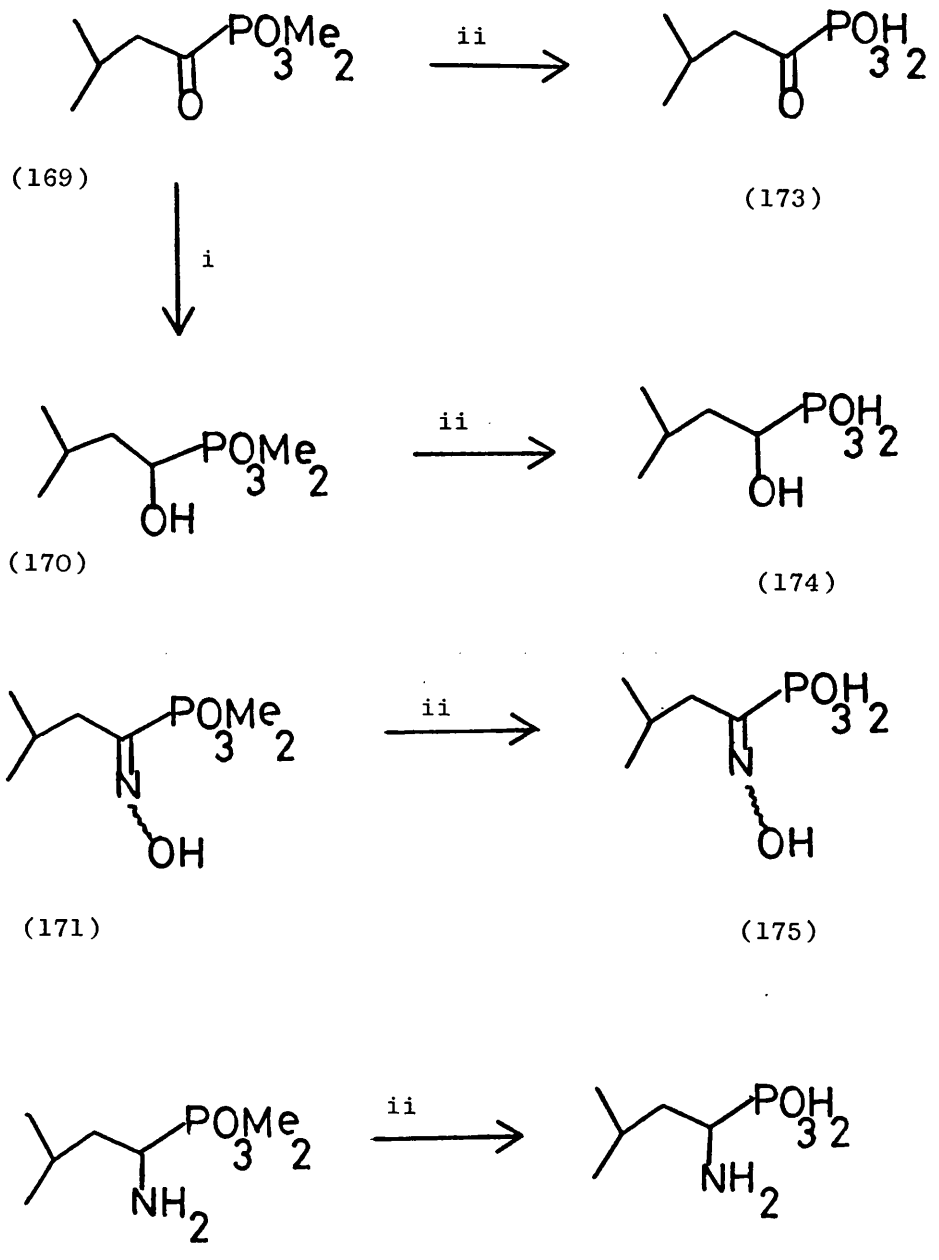
6. The synthesis of some novel phosphonic acids.

The aminophosphonic analogue of leucine, 1-amino-3-methyl butyl phosphonic acid was prepared by adapting a route first utilised by Japanese workers[185] (Scheme 55).



i, $P(OMe)_2$; ii, $NH_2OH.HCl$; iii, H_2/Raney-Ni; iv, conc. HCl.

(Scheme 55).



i, $NaBH_4$; ii, TMSBr, H_2O/Acetone 1:9

(Scheme 56).

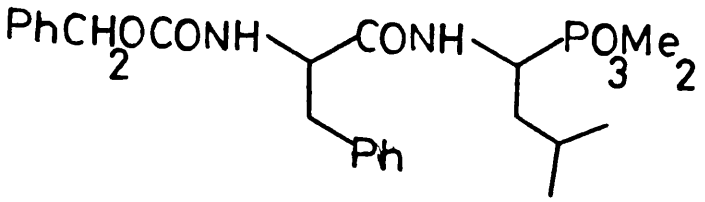
As many simple phosphonic acids such as phosphonomycin are of biological interest, it was postulated that phosphonic acids derived from the intermediates shown in Scheme 55 might also exhibit useful biological activity.

The facile dealkylation of phosphonic acid dialkyl esters using bromotrimethylsilane (TMSBr) has been demonstrated by McKenna and co-workers[186].

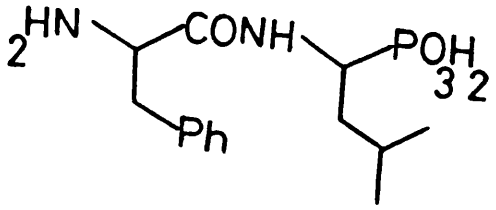
We prepared phosphonic acids (173) to (175) from the corresponding dimethyl phosphonates (169) to (172) by treatment with excess bromotrimethylsilane (TMSBr) followed by hydrolysis of the trimethylsilyl esters (Scheme 56). Compound (173) showed ^{1}H NMR resonances at δ3.78 (CH_2CO), 2.2 (CH), 0.94 ($(CH_3)_2$). The IR spectrum showed characteristic stretches at 3300-2700 (P-OH), 1710 (C=O), 1030 (P=O) cm^{-1}. The MS of (173) showed m/z 166 (M^+). Compounds (174) and (175) were characterised as for (173) above.

We also prepared the dipeptide analogue (176) *via* the activation of N-benzyloxycarbonyl phenylalanine using diphenyl phosphinyl chloride (DPPCl) and coupling this mixed anhydride with 1-amino-3-methylbutylphosphonic acid dimethyl ester (172). The structure of the coupled product was proven by NMR, IR and MS spectra. The N-benzyloxycarbonyl and phosphonate dimethyl ester groups were removed using hydrogen bromide in glacial acetic acid to yield (177). Compound (177) showed characteristic resonances in the ^{1}H NMR at δ 7.31 (aromatic), 3.2 (CH_2Ph), 0.8 ($(CH_3)_2$). The CMR of (177) indicated signals at δ 169.9 (C=O),

134-127 (aromatic), 52.98 and 51.42 (CHP, J_{CP} 110 Hz), 20.64 ($(CH_3)_2$). FAB MS on (197) showed m/z 315 (m+1) and 313 (m-1).



(176)



(177)

The phosphonic acids (173) to (177) were submitted for broad spectrum antibacterial screening. No significant biological activity was observed. Compound (176) may be of interest as a possible key intermediate in the synthesis of exophosphono-enkephalins. These have been demonstrated to show interesting properties at opoid receptors.[168]

b) Summary

Some novel phosphosphonic acids have been synthesised and their antibiotic activities assessed. Compounds have been prepared which may be further modified to molecules of greater biological potential.

EXPERIMENTAL

Melting points were determined using an Electrothermal Mk. II apparatus and are uncorrected. I.r. spectra were recorded with Perkin-Elmer 197 and 1310 grating spectrophotometers. ^{1}H n.m.r. spectra were recorded at 60 MHz with Perkin-Elmer R24B and Varian EM 360 spectrometers and at 100MHz with a JEOL PS 100 spectrometer. 400MHz n.m.r. spectra were recorded using the facility at the University of Warwick. ^{31}P n.m.r. spectra were recorded at 40.5MHz with a JEOL PS 100 spectrometer and ^{13}C n.m.r. spectra were recorded at 22.5MHz with a JEOL FX 90 Q spectrometer. U.V. spectra were obtained using Perkin-Elmer 402 and Lambda 3 spectrometers. Mass spectra were recorded on VG 7070E with VG 2000 data system, high resolution mass spectra were recorded on this system and by the Physical and Chemical Measurements Unit (Harwell).

Reactions were monitored by t.l.c. on Merck DC-Alufolien Kieselgel 60 F_{254} using an appropriate eluent. Column chromatography was carried out using pressurised short-path columns with Kieselgel 60 F_{254} Separation of small quantities was achieved by means of preparative t.l.c. on Merck PSC-Fertigplatten coated with Kieselgel 60 F_{254}.

Since reactions often produced complex mixtures it was found advantageous to use complementary methods of product detection.

(a) Ultraviolet irradiation of t.l.c. plates impregnated with a fluorescent indicator.

(b) Iodine vapour was used as a general but indiscriminating reagent.

(c) A 5% ethanolic solution of anisaldehyde with a trace of acetic acid and concentrated sulphuric acid and heating was often used.

(d) A 5% Ammonium Molybdate in 1M sulphuric acid was found to be extremely sensitive but indiscriminating.

(e) A ninhydrin solution containing 0.3g of ninhydrin in 100 ml of butan-1-ol and 3 ml of glacial acetic acid.

Amino-acid analysis was carried out using a Perkin-Elmer 6AH amino-acid analyser.

Solvents

Petroleum ether refers to the fraction of b.p. 40 - 60^{o}C and ether refers to diethyl ether. Other solvents were reagent grade unless otherwise stated. Solvents used for H.P.L.C. were ANALAR Grade. Solvents used for chromatography were redistilled and reaction solvents were generally dried by an appropriate procedure.

H.P.L.C. Studies

H.P.L.C. Studies were carried out using Spherisorb 5 ODS 25cm x 4mm i.d. as the stationary phase. A dual piston LDC Consta Metric pump and LDC Consta Metric spectromonitor detector were used. Traces were recorded using a Phillips PM 3252 dual pen recorder and peak areas measured using a Hewlett Packard 3390A integrator.

Preparation of aminobenzyl phosphonic acid

Freshly distilled benzaldehyde (7.62 ml, 0.075 mol) was added at room temperature over 20 mins. to a stirred mixture of benzyl carbamate (7.56 g, 0.05 mol), phosphorus trichloride (4.35, 0.05 mol) and glacial acetic acid (10 ml). The mixture was heated at reflux for 40 min., treated with 4N hydrochloric acid (50 ml) and refluxed for a further 30 min. After cooling, the organic layer was removed and the aqueous layer purified by boiling with activated charcoal (2 g). The solution was filtered and evaporated to dryness in vacuo. The residue was then dissolved in methanol (35 ml) and treated with 1,2-epoxypropane until pH 6-7 was reached. The precipitated product was washed with acetone and recrystallised from methanol/water (1:2). This yielded a white solid (5.7 g, 62%) m.p. 278-280°C. (Lit.[106] 280-282°C).

Preparation of N-benzyloxycarbonyl-aminobenzylphosphonic acid

Aminobenzyl phosphonic acid (2 g, 10.6 mmol) in water (12 ml) was added to benzyloxycarbonyl chloride (2.46 g, 16 mmol) in ether (12 ml). The stirred mixture was kept between pH 9-9.5 by addition of 4M NaOH over a period of 9 hrs. The mixture was stirred for a further 8 hrs. The ether layer was discarded and the aqueous phase was poured onto water (30 ml), concentrated sulphuric acid (30 ml) and ice (100 g). This was then exhaustively extracted with ether. The ether was removed in vacuo to yield a white solid (1.35 g, 42%).

Attempted Coupling of N-benzyloxycarbonyl-aminobenzylphosphonic acid with phenylglycine methyl ester. Identification of product (125)

N-Benzyloxycarbonyl aminobenzylphosphonic acid (0.94 g, 3 mmol) in dimethyl formamide (50 ml) was treated with freshly distilled pivoyl chloride (0.73 g, 6 mmol) and N-methyl morpholine (0.61 g 6 mmol) at -23°C. The mixture was stirred at -23°C for 1 hr. Phenylglycine methyl ester hydrochloride (0.51 g, 3 mmol) and N-methyl horpholine (0.61 g, 6 mmol) were added to the stirred mixture. The reaction mixture was allowed to warm to room temperature and stirred for 8 hrs. The solvent was removed in vacuo and the resulting residue dissolved in chloroform and washed with water. The organic solution was dried over magnesium sulphate, filtered and the solvent removed in vacuo to yield a yellow oil. Chromatography on silica, eluting with ethyl acetate, yielded a colourless gum (1.23 g) This was shown to be N-tert butyloxyphenylglycine methyl ester (125). δ_H (100MHz:$CDCl_3$) 1.2 (9H, s, $(CH_3)_3$), 3.64 (3H, s, OCH_3), 5.46 (1H, s, CH), 6.66 (1H, br d, NH), 7.2 (5H, m, aromatic); δ_C ($CDCl_3$) 27.5 ($\underline{C}H_3$), 38.7 ($-\underline{C}(CH_3)_3$), 56.5 ($\underline{C}H$), 52.6 ($O\underline{C}H_3$), 127.20, 128.45, 128.99, 136.95 (aromatic), 171 ($\underline{C}ONH$), 177 ($\underline{C}O_2Me$); m/z 249 (M^+).

1-Hydroxymethyl phthalimide

A suspension of phthalimide (102.2 g, 0.7 mol) and 40% formaldehyde (52 ml) in water (350 ml) was heated at reflux until the solution turned clear. The mixture was heated at reflux for a further 1 hr and then cooled to room temperature. The solution was kept at 0°C overnight and a white solid crystallised from the solution. The resulting solid was filtered, washed with water and recrystallised from methanol/water to yield a white crystalline material (113.9 g, 92%) m.p. 138-140°C (Lit.[46] 137-141°C).

1-Bromomethyl phthalimide

A suspension of hydroxymethylphthalimide (30.6 g, 0.2mol) in chloroform (300ml) was stirred and heated gently at reflux. Dry hydrogen bromide was bubbled through the stirred mixture via a submerged glass inlet at a moderate rate for 2 hrs. After a short period (20 min.) the mixture became homogeneous and eventually turned orange. The solution was poured (whilst still warm) into a separating funnel and the lower aqueous hydrogen bromide layer discarded. The organic layer was washed with warm water (2 x 200 ml). The organic solution was dried over magnesium sulphate, filtered through celite and the solvent removed in vacuo to yield a white crystalline solid. Recrystallisation from chloroform yielded a colourless crystalline material (24.45 g, 68%) m.p. 149-150°C. (Lit.[46] 148°C).

Dimethyl Phthalimidomethyl phosphonate

Bromomethyl phthalimide (23 g, 0.096 mol), freshly distilled trimethyl phosphite (12.45 ml, 0.096 mol) and redistilled xylene (200 ml) were heated gently under nitrogen for 30 mins. The temperature was gradually raised to reflux and the mixture heated at reflux for 5 hrs. Some of the solvent (150 ml) was then distilled away, and the remaining solution was allowed to cool overnight. The crystalline product was washed with ether and dried in vacuo to yield a white solid (24.44 g, 91%). m.p. 117-118°C (Chloroform-carbon tetrachloride) Lit. m.p 117.5-119°C.

O-Methyl phthalimidomethyl phosphosphonic acid (135)

Dimethyl phthalimidomethyl phosphonate (2.69 g, 10 mmol), in dry toluene (20 ml) was heated at reflux with phosphorus pentachloride (2.08 g, 10 mmol) for 5 hrs. The solvent was removed in vacuo and the resulting solid stirred overnight with acetone/water 9:1 (20 ml). The solvents were removed under reduced pressure and the resulting solid washed with chloroform to yield a white solid. (1.99 g, 78%); m.p. 142-144°C (from methanol); (Found: C, 47.05, H, 3.95; N 5.49; P, 12.14. $C_{10}H_{10}NO_5P$ requires C, 46.95; H, 4.13; N, 5.45; P, 11.98). ν_{max} (Nujol) 3500-2700 (P-OH), 1780, 1720 (C=O), 1600 (aromatic), 1215 (P-OCH_3), 1020 (P=O); δ_H (60MHz:$(CD_3)_2SO$) 3.6 (3H, d, J_{PH} 11Hz, $POCH_3$), 3.9 (2H, d, J_{PH} 11Hz, CH_2P), 6.7 (1H, br s, POH), 7.8 (4H, m, aromatic); δ_C($(CD_3)_2SO$) 24.5 and 31.2 ($\underline{C}H_2P$, J_{CP} 149Hz), 53.62 and 53.92 ($PO\underline{C}H_3$, J_{CP} 6.1Hz), 116.96, 125.5, 128.4 (aromatic), 160.5 ($\underline{C}$=O); m/z (M+1) 256 [+] ion FAB (M-1) 254 [-] ion FAB.

Attempted preparation of N-[[[(phthaloyl)amino]methyl] methoxyphosphinyl]-L-phenylalanine methyl ester.

Isolation of N-diethoxyphenyl phenylalanine methyl ester (129).

O-Methyl phthalimidomethyl phosphonic acid (510 mg, 2 mmol) was treated with diethyl chlorophosphate (0.3 ml, 2 mmol) and N-methyl morpholine (0.2 ml, 2 mmol) in dry tetrahydrofuran at -20°C. The mixture was stirred at -20°C for 30 mins. Phenylalanine methyl ester hydrochloride (430 mg, 2 mmol) and N-methyl morpholine (0.2 ml, 2 mmol) were added to the stirred mixture. The reaction mixture was allowed to warm to room temperature and stirred for 8 hrs. Insoluble materials were filtered and the solvent removed in vacuo to yield a yellow gum. Chromatography on silica eluting with ethyl acetate yielded a colourless gum (610 mg). This was shown to be N-diethoxyphosphonyl phenylalanine methyl ester (129).

δ_H (100MHz:$CDCl_3$) 1.25 (6H, m, $OCH_2\underline{CH_3}$), 3.05 (2H, br m, $\underline{CH_2}Ph$), 3.67 (3H, s, OCH_3), 3.9 (5H, m, $PO\underline{CH_2}$ and CH), 7.25 (5H, m, aromatic) 7.8 (1H, br, NH); δ_C ($CDCl_3$) 15.98, 16.31 ($\underline{CH_3}$), 40.58, 40.85 ($\underline{CH_2}Ph$) 52.06 (OCH_3), 55.85 (CH), 62.41 and 62.63 ($PO\underline{CH_2}$, J_{CP} 4.78Hz), 123.63, 127.04, 128.56, 129.56, 136.36 (aromatic), 173.09 ($\underline{C}=O$).

N-[[[(Phthaloyl)amino]methyl]methoxyphosphinyl]-L-phenylalanine methyl ester (136).

Phthalimidomethyl dimethyl phosphonate (19.51 g, 0.0725mmol) in dry toluene (150 ml) was heated at reflux with phosphorus pentachloride (16.61 g, 1.1 eq) for 5 hrs. The solvent was removed in vacuo and the oily residue was dissolved in dry ethanol free chloroform (50 ml). This was added dropwise to a chloroform solution of L-phenylalanine methyl ester hydrochloride (15.09 g, 0.07 mol) and triethylamine (21.18 ml , 2.1 eq) at 0°C. The solution was allowed to warm to room temperature and stirred for 8 hrs. The mixture was diluted with chloroform (50 ml) and washed with 1M sodium dihydrogen phosphate (2 x 150 ml), saturated sodium bicarbonate (2 x 100 ml) and water (2 x 100 ml). The organic solution was dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a yellow oil. Chromatography on silica eluting with petroleum ether/ ethyl acetate 1:1 yielded a white solid (6.87 g, 23%). (Found: C, 56.7; H, 5.0; N, 6.6; P, 6.9. $C_{20}H_{21}N_2O_6P.\frac{1}{2}H_2O$ requires C, 56.5; H, 5.2; N, 6.6; P, 7.3); ν_{max} ($CHCl_3$) 3400 (NH), 2950 (CH), 1780, 1720 (C=O), 1600 (aromatics), 1380 (OCH_3), 1250 (P-OCH_3), 1045 (P=O) cm^{-1}; δ_H (400 MHz; $CDCl_3$) 3.02-3.12 (2H, m, CH_2Ph), 3.41 (1H, br t, P-NH), 3.61 (3H, d, J_{PH} 11Hz, $POCH_3$), 3.68 (3H, s, CO_2CH_3), 3.7 (1H, dd, J_{PH} 11Hz, CH_A-P), 3.95 (1H, dd, J_{PH} 11Hz, CH_BP), 4.4 (1H, m, $\underline{C}HCH_2Ph$), 7.2 (5H, m, Ph), 7.8-7.9 (4H, m, F+); δ_P ($CDCl_3$) 23 (1P, m); δ_C ($CDCl_3$) 32.13 and 38.41 ($\underline{C}H_2P$, J_{CP} 141.6 Hz), 40.47 ($\underline{C}H_2Ph$), 52.17 ($O\underline{C}H_3$), 52.98 ($\underline{C}HCH_2$), 54.72 and 54.93 (P-$O\underline{C}H_3$, J_{CP} 5.8Hz), 123.41, 126.88, 128.40, 129.53, 134.14, 131.91, 136.30 (aromatics), 157.18 ($\underline{C}O_2CH_3$), 173.19 (-$\underline{C}$ONH); m/z 417 (M+1), 357, 325, 269, 238, 160 (base).

N- [[(Phthaloyl)amino]methyl]methoxyphosphinyl]-L-glycine methyl ester (140).

Phthalimidomethyl phosphonic acid (138) (122 mg, 0.51 mmol) was heated at reflux with excess thionyl chloride (5 ml) for 48 hrs. The excess thionyl chloride was removed in vacuo. Traces of thionyl chloride remaining were removed by dissolving the residue in dry ethanol-free chloroform and removing the solvent under reduced pressure. The resulting white solid was dissolved in dry ethanol free chloroform and stirred at $0^{o}C$ with glycine methyl ester hydrochloride (58.91 mg, 0.51 mmol) and triethylamine (103 mg, 1.02 mmol). The stirred solution was allowed to warm to room temperature and stirred for a further 5 hrs. To the stirred mixture dry methanol (1 ml) and triethylamine (52 mg, 0.51 mmol) were added. The reaction mixture was stirred for a further 8 hrs; washed with water (2 x 5 ml), dried over magnesium sulphate, filtered and the solvent removed in vacuo to yield a white paste. Chromatography on silica eluting with chloroform/ethanol (9:1) yielded a white paste (27 mg, 16%). (Found: M^{+} 326.0651 $C_{13}H_{15}N_2O_6P$ requires M, 326.0668); ν_{max} ($CHCl_3$) 3370 (NH), 2950 (CH), 1780, 1710 (CONCO), 1750 (CO_2Me), 1410 (OMe), 1220 (P-OCH_3), 1140 (P=O) cm^{-1}; δ_H(100MHz:$CDCl_3$) 3.73 (2H, s, CH_2), 3.76 (3H, s, CO_2CH_3), 3.82 (3H, d, J_{PH} 11 Hz, P-OCH_3), 4.10 (2H, d, J_{PH} 11Hz, CH_2P), 4.42 (1H, br, NH), 7.80 (4H, m, aromatic); m/z 326 (M^{+}), 294,238, 160 (base).

N-Benzyloxycarbonylglycyl-aminomethyl(methoxyphosphinyl)-L-phenylalanine methyl ester (142).

Crude aminomethyl(methoxyphosphinyl)-L-phenylalanine methyl ester (510 mg, 1.8 mmol) in dry tetrahydrofuran (25 ml) was added to a stirred solution of O-benzyl-N-benzyloxycarbonyl-glycine (455 mg, 2 mmol) and dicyclohexyl carbodiimide (DCC) (0.5 g, 2.16 mmol) in tetrahydrofuran (25 ml) at $0^{o}C$. The mixture was allowed to warm to room temperature and stirred for 72 hrs. The precipitated dicyclohexyl urea was filtered and the solvent removed in vacuo. The resulting oil was dissolved in ethyl acetate (25 ml) and washed with saturated sodium bicarbonate (2 x 20 ml), water (2 x 20 ml) and saturated sodium chloride (2 x 20 ml). The organic solution was dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a pale yellow oil. Chromatography on silica eluting with ethyl acetate yielded (142) as a colourless gum (485 mg, 56%) (Found: C, 54.44; H, 5.97; N, 8.78. $C_{22}H_{28}N_3O_7P.\frac{1}{2}H_2O$ requires C, 54.27; H, 6.01; N, 8.64%); ν_{max} ($CHCl_3$) 3400 (NH), 2950 (CH), 1690-1750 (C=O), 1600 (aromatic), 1450 (OCH_3), 1200 (P-OCH_3), 1050 (P=O)cm^{-1}; δ_H (400MHz:$CDCl_3$) 2.83 (1H, m, $C\underline{H}_A H_M Ph$), 3.08 (1H, m, $CH_A\underline{H}_M Ph$), 3.19 (3H, d, J_{PH} 11.1Hz, P-OCH_3), 3.28 (1H, m, $C\underline{H}_A H_B P$), 3.65 (1H, m, $CH_A\underline{H}_B P$), 3.72 (3H, s, $CO_2C\underline{H}_3$), 4.05 (1H, octet, $C\underline{H}_X CH_A CH_M Ph$), 4.16 (1H, t, P-NH), 5.10 (2H, s, $OC\underline{H}_2Ph$), 6.05 (1H, br t $OCON\underline{H}$), 7.15-7.33 (10H, m, aromatic), 7.58 (1H, br t, CONH); δ_C ($CDCl_3$), 34.93 and 36.40 ($\underline{C}H_2P$, J_{CP} 146.92Hz), 40.28, 40.33 ($\underline{C}H_2Ph$), 44.13 ($\underline{C}H_2CO$), 50.64 and 50.57 (P-$O\underline{C}H_3$, J_{CP} 6.73Hz), 52.35 ($CO_2\underline{C}H_3$), 55.21 ($\underline{C}HCH_2Ph$), 66.82 ($\underline{C}H_2OPh$), 126.97, 127.9, 128.3, 128.49, 129.37, 136.18, 136.38 (aromatic), 156.33 ($O\underline{C}ONH$), 169.29, 169.34 ($\underline{C}O_2Me$), 173.68 ($\underline{C}ONH$); m/z, 500 (M+Na), 478 (M+1) [+] ion FAB.

O-Benzyl-N-benzyloxycarbonyl-L-tyrosyl-glycyl-aminomethyl (methoxyphosphinyl)-L-phenylalanine methyl ester (143)

Crude aminomethyl(methoxyphosphinyl)-L-phenylalanine methyl ester (510 mg, 1.8 mmol) in dry tetrahydrofuran (25 ml) was added to a stirred solution of O-benzyl-N-benzyloxycarbonyl-tyrosyl-glycine (800 mg, 2 mmol) and dicyclohexylcarbodiimide (DCC) (500 mg, 2.16 mmol) in tetrahydrofuran (25 ml) at $0^{o}C$. The mixture was allowed to warm to room temperature and stirred for 72 hrs. The precipitated dicyclohexylurea was filtered and the solvent removed in vacuo. The resulting oil was dissolved on ethyl acetate (25 ml) and washed with saturated sodium bicarbonate (2 x 20 ml), water (2 x 20 ml) and saturated sodium chloride (2 x 20 ml). The organic solution was dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a pale yellow oil. Chromatography on silica eluting with ethyl acetate yielded a foam (730 mg, 55%). (Found: C, 61.87; H, 5.99; N, 7.93. $C_{38}H_{43}N_4O_9P.\frac{1}{2}H_2O$ requires C, 61.68; H, 5.99; N, 7.58%); ν_{max} ($CHCl_3$) 3370 (NH), 2850 (CH), 1720, 1640 (C=O), 1610 (aromatic), 1480 (OCH_3) 1250 ($P-OCH_3$), 1050 (P=O)cm^{-1}; δ_H (400MHz:$(CD_3)_2SO$) 2.98 (2H, m, $CH_{\underline{A}}H_MPh$ and $CH_{\underline{A}}'H_M'Ph$), 3.16 (2H, m, $CH_A\underline{H}_MPh$ and $CH_A'\underline{H}_M'Ph$), 3.30 (2H, m, CH_2P), 3.38 (3H, d, J_{PH} 11.1 Hz, $P-OCH_3$), 3.61 (3H, s, $CO_2\underline{CH}_3$), 3.77 (2H, m, CH_2CO), 4.15 (1H, octet, $\underline{CH}_XCH_2Ph$, 4.40 (1H, octet, $\underline{CH}_X'Tyr$), 4.93 (2H, q, $Ph\underline{CH}_2O$, J 11.8Hz), 4.98 (2H, s, $PhO\underline{CH}_2$), 6.81 (2H, dd, J_{AX} 8.61Hz), 7.13 (2H, dd, J_{XA} 8.59Hz), 7.15-7.31 (15H, m, aromatic), 7.55 (1H, t d, $N\underline{H}CH_2P$), 8.10 (1H, t, $N\underline{H}CH_2$);

δ_C ($(CD_3)_2SO$) 35.23 and 35.57 ($\underline{C}H_2P$, J_{CP} 146.15Hz), 40.78 ($\underline{C}H_2Ph$), 48.71 and 48.78 (P-$O\underline{C}H_3$, J_{CP} 6.91Hz), 50.17 ($NH\underline{C}H_2CO$), 53.57 ($CO_2\underline{C}H_3$), 54.87 ($\underline{C}HCH_2Ph$), 64.15 ($Ph\underline{C}H_2OCO$), 67.86 ($Ph\underline{C}H_2O$), 112.78, 124.94, 125.75, 125.88, 126.03, 126.55, 126.68, 127.75, 128.30, 128.57, 135.10, 135.47, 154.37 (aromatic), 155.48 ($O\underline{C}ONH$), 166.99 ($\underline{C}O_2CH_3$), 170.21, 171.61 ($\underline{C}ONH$); m/z 753 (M+Na), 731 (M+1) [+] ion FAB.

N-[[[(Phthaloyl)amino]methyl]methoxyphosphinyl]-L-phenylalanyl-L-Leucine methyl ester (144)

Phthalimidomethyl dimethylphosphonate (2.1g, 7.8 mmol) in dry toluene (50 ml) was stirred with phosphorus pentachloride (1.8 g, 1.1 eq). The mixture was heated at reflux for 5 hrs. The solvent was removed in vacuo to yield a yellow gum. This was dissolved in dry tetrahydrofuran and added to a stirred solution of phenylalanyl leucine methyl ester hydrochloride (2.55 g, 7.8 mmol) and N-methyl morpholine (1.9 ml, 2.1 eq). The mixture was allowed to warm to room temperature and stirred for 8 hrs. The precipitated N-methyl morpholine hydrochloride was removed by filtration. The solvent was removed in vacuo to yield a yellow oil. The oil was dissolved in ethyl acetate and washed with water (2 x 40 ml), 5% citric acid (2 x 50 ml), 5% sodium bicarbonate (2 x 50 ml) and water (1 x 50 ml). The solution was dried over magnesium sulphate, filtered, and the solvent removed in vacuo to yield a yellow oil. Chromatography on silica eluting with ethyl acetate yielded a pale yellow gum (1.31 g, 32%) which solidified on standing. $[\alpha]_D^{20}$ MeOH 35.5°; (Found: C, 58.5; H, 6.1; N, 8.0. $C_{26}H_{32}N_3O_7P$ requires C, 58.94; H, 6.09; N, 7.93); ν_{max} ($CHCl_3$) 3370 (NH), 2950 (CH), 1780 (CONCO), 1750 (CO_2Me), 1730 (CONCO), 1650 (CONH), 1620 (aromatic), 1400 (OCH_3), 1250 (P-OCH_3), 1050 (P=O)cm^{-1}; δH (400MHz:$CDCl_3$) 0.86 (3H, br t, CH_3), 0.91 (3H, br t, CH_3), 1.57 (2H, br d, -$CH\underline{H}_2$), 3.10 (1H, m, $C\underline{H}_B H_A Ph$), 3.15 (1H, m, $CH_A \underline{H}_B Ph$), 3.42 (1H, dd, P-NH), 3.62 (1.8H, s, CO_2Me), 3.66 (1.2H, s, CO_2Me) minor diasteroisomer. 3.48 (1.2H, d, J_{PH} 11Hz, P-OCH_3), 3.80 (1.8H, d, J_{PH} 11Hz, $POCH_3$), 3.85 (0.4H, dd, J_{PH} 11Hz, $CH_{A\underline{H}B}P$), 3.95 (0.6H, dd J_{PH} 11Hz, $CH_{A\underline{H}B}P$),

4.05 (0.4H, dd, J_{PH} 11Hz, $C\underline{H}_A H_B P$), 4.15 (0.6H, dd J_{PH} 11Hz, $C\underline{H}_A H_B P$), 4.25 (1H, m, NHC$\underline{H}$), 4.55 (1H, m, NH$\underline{C}$H), 7.3 (5H, m, aromatic), 7.65 (1H, br, CON$\underline{H}$) 7.72 (2H, m, aromatic), 7.80 (2H, m, aromatic); δ_C ($CDCl_3$) 22.78, 22.81 ($\underline{C}H_3$), 24.70 ($\underline{C}HMe_2$), 32.29 and 40.03 (CH_2P, J_{CP} 174.56Hz), 40.19 ($\underline{C}H_2CH$), 40.96 ($\underline{C}H_2Ph$), 50.92 ($\underline{C}HCH_2$), 51.81 and 51.57 (P-O$\underline{C}H_3$, J_{CP} 6.1Hz), 52.11 (O$\underline{C}H_3$), 56.12 ($\underline{C}HCH_2Ph$), 123.57, 126.93, 128.56, 129.69, 131.86, 134.30, 136.68 (aromatic), 167.40 ($\underline{C}O_2Me$), 171.84, 172.06 (CONCO), 173.14 (CONH); m/z 530 (M+1), 498 (M-OMe), 293 (base).

O-Benzyl-N-benzyloxycarbonyl-L-tyrosyl-glycyl)-aminomethyl (methoxyphosphinyl)-L-phenylalanyl-L-leucine methyl ester (145).

O-Benzyl-N-benzyloxycarbonyltyrosyl-glycine (780 mg, 1.7 mmol) in dry dimethyl formamide (40 ml) under argon at -25°C was treated with diphenylphosphinyl chloride (DPPCl) (0.4 g, 1.7 mmol). The mixture was stirred at -25°C for 15 mins. A dimethyl formamide solution containing crude aminomethyl(methoxyphosphinyl) -L-phenylalanine methyl ester (700 mg) was added to the stirred solution at -25°C. N-Methyl morpholine (0.19 ml, 1.7 mmol) was added to the stirred mixture. The mixture was allowed to warm to room temperature and stirred for 8 hrs. The solvent was removed in vacuo and the resulting oil dissolved in ethyl acetate (75 ml). The organic solution was washed with water (2 x 25 ml), 5% citric acid (2 x 25 ml), 0.5M potassium carbonate (2 x 25 ml), water (2 x 25 ml) and saturated sodium chloride (2 x 25 ml). The organic solution was dried over sodium sulphate, filtered and the solvent removed in vacuo to yield (145) as a colourless gel (540 mg, 34%). Preparative thin layer chromatography eluting with chloroform/methanol (95:5) yielded a colourless foam. Amino acid analysis, glycine 1.01, leucine 0.92, tyrosine 0.97, phenylalanine 0.91; δ_H (400MHz:$(CD_3)_2SO$) 0.83 (6H, m, $(CH_3)_2$), 1.55 (2H, m, $CHC\underline{H}_2$), 1.62 (1H, m, $CH_2C\underline{H}$), 2.98 (2H, m, $CH_{m-x}\underline{H}_x Ph$ and $CH_m{}'\underline{H}_x{}'Ph$), 3.14 (2H, m, $CH_m\underline{H}_x Ph$ and $CH_m{}'H_x{}'Ph$), 3.54 (1H, dd, P-NH), 3.58 (3H, d, J_{PH} 11Hz, P-OCH_3), 3.64 (3H, s, $CO_2C\underline{H}_3$), 3.82 (2H, br m, $NHC\underline{H}_2CO$), 3.85 (1H, dd, J_{PH} 11Hz, $CH_A\underline{H}_BP$), 4.00 (1H, dd, J_{PH} 11Hz, $CH_A\underline{H}_BP$), 4.15 (1H, m, $C\underline{H}CH_2$), 4.55 (2H, m, $C\underline{H}_ACH_2Ph$ and $C\underline{H}_A{}'Tyr$), 4.98 (2H, s, $PhOC\underline{H}_2$),

5.02 (2H, q, $C\underline{H}_2OCO$), 5.60 (1H, br, NHCO), 7.15-7.42 (15H, m, aromatic), 6.85 (2H, dd, J_{AX} 8.6Hz), 7.05 (2H, dd J_{XA} 8.6Hz), 7.90 (1H, br, $N\underline{H}CH_2CO$); m/z 866 (M+Na), 844 (M+1) [+] ion FAB.

Attempted deprotection of O-benzyl-N-benzyoxycarbonyl-L-tyrosyl-glycyl-aminomethyl(methoxyphosphinyl)-L-phenylalanyl-L-leucine methyl ester.

a) The protected pentapeptide (145) (175 mg, 0.2 mmol) in methanol (10 ml) was stirred with ammonium formate (50 mg) and 10% Palladium on charcoal (10 mg). After 5 mins the starting material had disappeared and a major product that sprayed positive with ninhydrin was present. This was shown by t.l.c. to be phenylalanyl leucine methyl ester.

b) The protected pentapeptide (145) (230 mg, 0.27 mmol) in dry dichloromethane was treated with bromotrimethylsilane (TMSBr) (5 eq, 0.21 ml). The mixture was stirred at room temperature for 8 hrs. The dichloromethane was removed in vacuo to yield a yellow gum which was dissolved in acetone/water (9:1) (20 ml) and the mixture stirred for 2 hrs. The solvents were removed in vacuo to yield a pale yellow gum. Reverse phase chromatography

eluting with methanol/water (70:30) yielded a colourless gum (50 mg). T.l.c. showed this gum to contain a major product that sprayed positive with ninhydrin, indicating a free amino function. ^{1}H n.m.r. showed this to be a mixture of products. No satisfactory mass spectral data was obtained for a structure to be proposed.

1-(1-Hydroxy-3-oxo-2H-isoindol-2-yl)methyl dimethyl phosphonate (157)

Phthalimidomethyl dimethyl phosphonate (269 mg, 1 mmol) was stirred at room temperature with sodium borohydride (21 mg, 1.1 mmol) in propan-2-ol (20 ml) and water (2 ml). The mixture was stirred at room temperature for 2 hrs. Acetic acid (2 ml) was added dropwise and when the foaming subsided the mixture was stirred at room temperature for a further 30 mins. Water (20 ml) was added to the mixture and the aqueous system extracted with dichloromethane (2 x 20 ml). The organic solution was dried over magnesium sulphate, filtered and the solvent removed in vacuo to yield a colourless oil. Chromatography on silica eluting with ethyl acetate yielded a colourless gum (212 mg, 79%). (Found: M^+ 271.0580 $C_{11}H_{14}NO_5P$ requires M, 271.0609); ν_{max} 3350 (OH), 1700 (C=O), 1230 (P-OCH_3), 1050 (P=O) cm^{-1}; δ_H (100MHz:$CDCl_3$) 3.75 (6H, 2 x d, J_{PH} 11Hz, P-OCH_3), 4.05 (2H, dd, J_{PH} 11Hz, CH_2P), 6.05 (1H, s, C<u>H</u>OH), 7.4-7.8 (4H, m, aromatics); δ_C ($CDCl_3$) 30.34- and 37.3 (CH_2P, J_{CP} 157.5 Hz), 52.93 and 53.25 (P-OCH_3, J_{CP} 7.33Hz), 53.09 and 53.36 (P-O<u>C</u>H_3, J_{CP} 6.11Hz), 81.9 (<u>C</u>HOH), 123.30, 123.57, 128.56, 129.59, 130.83, 132.51 (aromatics), 167 (C=O); m/z 271 (M^+), 162, 133 (base).

1-(1-Hydroxy-3-oxo-2H-isoindol-2-yl)methyl -(methoxyphosphinyl-L-phenylalanyl-L-leucine methyl ester (158).

N-[[[(Phthaloyl)amino]methyl]methoxyphosphinyl]-L-phenylalanyl-leucine methyl ester (300 mg, 0.57 mmol) was stirred at room temperature with sodium borohydride (12 mg, 0.63 mmol)

in propan-2-ol (20 ml) and water (2 ml). The mixture was stirred at room temperature for 2 hrs. Acetic acid (2 ml) was added dropwise and when foaming subsided the mixture was stirred at room temperature for a further 30 mins. Water (20 ml) was added to the mixture and the aqueous system extracted with dichloromethane (2 x 20 ml). The organic solution was dried over magnesium sulphate, filtered and the solvent removed in vacuo to yield a colourless oil. Chromatography on silica eluting with ethyl acetate yielded a colourless gum (265 mg, 87%); ν_{max} ($CHCl_3$) 3350 (OH), 1680-1700 (CONH), 1740 (CO_2CH_3), 1250 (P-OCH_3), 1050 (P=O) cm^{-1}; δ_H (100MHz:$CDCl_3$) 0.85 (6H, br, $CH(CH_3)_2$), 1.48 (3H, br, CH_2CH), 3.08 (2H, m, CH_2Ph), 3.43 (2H, dd, J_{PH} 11Hz, CH_2P), 3.62 (3H, s, CO_2CH_3), 3.65 (3H, d, J_{PH} 11Hz, P-OCH_3), 3.8 (2H, m, NHCHCO), 3.45 (br, OH), 5.9 (1H, d, CHOH), 7.0-7.8 (9H, m, aromatic) δ_C ($CDCl_3$) 21.72, 22.81 ($(CH_3)_2$), 24.70 ($CH(CH_3)_2$), 29.68 and 34.13 (CH_2P, J_{CP} 100Hz), 40.52 (CH_2CH), 41.01 (CH_2Ph), 50.97 (NHCHCO) 51.03 and 51.36 (P-OCH_3, J_{CP} 7.1Hz), 52.23 (CO_2CH_3), 56.29, 56.67 (NHCHCO), 82.45, 82.72 (CHOH), 123.36, 123.57, 126.99, 128.26, 128.72, 129.59, 129.80, 131.05, 132.46, 136.79, 144.38, 144.48 (aromatic), 167.62 (CO_2Me), 173.25 (CONH); m/z 532 (M+1) [+] ion FAB, 530 (M-1) [-] ion FAB.

Deprotection of N,N'(2,2'-dibenzyl-1-aminomethyl)dimethyl phosphonate (159)

N,N'(2,2'-Dibenzyl-1-aminomethyl)dimethyl phosphonate (400 mg) in 4.4% formic acid in methanol (5 ml) was added to palladium black (400 mg) in 4.4% formic acid in methanol (10 ml). The mixture was continually stirred under an argon atmosphere for 15 hrs. The catalyst was filtered and washed with water (5 ml) and methanol (5 ml). The solvents were removed in vacuo to yield a colourless oil. The oil was redissolved in methanol/water 1:1 and put in the freezer. A product shown to be (160) crystallised out (110 mg, 70%). δ_H(100MHz:D_2O) 3.10 (2H, d, J_{PH} 12Hz, $\underline{C}H_2P$), 3.58 (3H, d, J_{PH} 10Hz, P-O$\underline{C}H_3$); $_C$ (D_2O) 31.91 and 38.30 ($\underline{C}H_2P$, J_{CP} 144Hz), 52.98 and 53.25 (P-O$\underline{C}H_3$, J_{CP} 6.1Hz); m/z [+] ion FAB 126 (M+1); [-] ion FAB 124 (M-1).

N,N'(2,2'-Diallyl-1-amimomethyl)dimethylphosphonate (161)

N,N'-Diallylamine (18.33 ml), dimethyl phosphite (24.7 ml, 1 eq) and 40% formaldehyde solution (40 ml) were stirred at room temperature for 10 hrs. Water and any unreacted starting materials were removed on a cold finger to yield a yellow oil. Bulb to bulb distillation yielded a yellow oil (40 g, 91%). b.p. 145°C at 0.2mm. (Found: M^+ 219.1024. $C_9H_{18}NO_3P$ requires M, 219.1024); ν_{max} (neat) 3100-2700 (CH), 1640 (C=C), 1440 (OCH_3), 1250 (P-OCH_3), 1050 (P=O) cm^{-1}; δ_H (100MHz; $CDCl_3$) 2.90 (2H, d, J_{PH} 12Hz CH_2P), 3.26 (2H, d, CH_2N), 3.78 (6H, d, J_{PH} 12Hz, $P(OCH_3)_2$), 5.2 (4H, m, $\underline{H}_2C=$), 5.75 (2H, m, =C$\underline{H}$-); δ_C ($CDCl_3$) 43.99 and 51.19 ($\underline{C}H_2P$, J_{CP} 162.35Hz), 52.5 and 52.77 (PO$\underline{C}H_3$, J_{CP} 6.1Hz), 57.91 and 58.29 ($\underline{C}H_2N$, J_{CP} 9.54Hz), 118.21 ($H_2\underline{C}=$), 135.0 (=$\underline{C}$H-); m/z 219(M^+), 204, 192, 110 (base), 68, 41, 28.

2-(Hydroxymethyl)-1-[H]-isoindol-1-oxo-3,(2H)-thione

Monothiophthalimide (150 mg, 0.92 mmol) in 40% aqueous formaldehyde (7.5 ml) and water (25 ml). The mixture was heated at reflux for 5 hrs and then extracted with ethyl acetate (2 x 20 ml) and the solvent removed in vacuo to yield a pink solid. Chromatography on silica eluting with dichloromethane/ethyl acetate (3:1) yielded a pink solid (145 mg, 82%) m.p. 117-119°C (from toluene). (Found: M^+ 193.0164 $C_9H_7NO_2S$ requires, M 193.0197); ν_{max} ($CHCl_3$) 3480 (OH), 1710 (C=O), 1600 (aromatic), 1350 (C=S); λ_{max} (EtOH) 327 (ε 5942), 290 (ε 7,971), 231 (ε 16,908); δ_H (60MHz:CD_3OD), 5.00 (1H, br s, OH), 5.32 (2H, s, CH_2), 7.65 (4H, m, aromatic); δ_C (CD_3OD) 63.71 ($\underline{C}H_2OH$), 123.9, 124.33, 124.81, 134.95, 135.59 (aromatic), 168.97 ($\underline{C}$=O), 198.44 ($\underline{C}$=S); m/z 193 (M^+), 163 (base), 103, 76, 28.

2-(Bromomethyl-1-[H]-Isoindole-1-oxo-3,(2H)-thione

2-(Hydroxymethyl)-1-[H]-Isoindole-1-oxo-3,(2H)-thione (120 mg, 0.62 mmol) in dry dichloromethane (15 ml) at room temperature was stirred with phosphorus tribromide (59 µl, 0.62 mmol) for 3 hrs. The organic solution was washed twice with water (2 x 5 ml) dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a pink solid. Chromatography on silica eluting with dichloromethane yielded a pink crystalline solid (120 mg, 76%) m.p. 109-111°C; (Found: M^+ 254.9355 $C_9H_6NOS^{79}Br$ requires M, 254.9353, Found: M^+ 256.9333 $C_9H_6NOS^{81}Br$ requires M, 256.9333); ν_{max} ($CHCl_3$) 3000 (CH), 1740 (C=O), 1600 (aromatic), 1350 (C=S), 1150 (C-Br)cm^{-1}; λ_{max} (EtOH) 324nm (ε 12,179), 286nm (ε 16,026), 231nm (ε 31,410); δ_H (60MHz:$CDCl_3$) 5.77 (2H, s, CH_2Br), 7.83 (4H, m, aromatic); δ_C ($CDCl_3$) 34.35 ($\underline{C}H_2$), 123.79, 124.55, 127.51, 134.30, 135.17, 137.93 (aromatic), 167.62 ($\underline{C}$=O), 194.27 ($\underline{C}$=S); m/z 257(M^+), 255 (M^+), 176 (base), 130.

1-(1,3-Dihydro-1-oxo-3-thio-2H-isoindol-2-yl)methyl dimethylphosphonate (162)

2-(Bromomethyl)-1-[H]-Isoindole-1-oxo-3-thio-2(H)-thione (100 mg 0.35 mmol) in dry xylene (10 ml) was heated at reflux with excess trimethylphosphite (3 ml) for 10 hrs. The solvent was removed in vacuo and the crude mixture chromatographed on silica eluting firstly with dichloromethane to remove any unreacted starting material. The product was eluted with dichloromethane/ethyl acetate (75:25). The solvents were removed to yield a pink crystalline solid m.p. 103-104°C. (Found: M^+ 285.0224. $C_{11}H_{12}NO_4PS$ requires M, 285.0235); ν_{max} ($CHCl_3$) 2950(CH), 1680 (C=O), 1600 (aromatics), 1400 (OCH_3), 1340 (C=S), 1200 (P-OCH_3), 1050 (P=O) cm^{-1}; λ_{max} (EtOH), 327nm (ε 7,143), 290nm (ε12,054), 231nm (ε33,929); δ_H (60MHz; $CDCl_3$) 3.37 (2H, d, J_{PH} 11Hz, CH_2P), 3.65 (6H, d, J_{PH} 11Hz, $P(OCH_3)_2$), 7.56 (4H, m, aromatic); δ_C ($CDCl_3$) 32.4 and 39.33 ($\underline{C}H_2P$), J_{CP} 157.25 Hz), 53.04 and 53.25 (P-$O\underline{C}H_3$, J_{CP} 4.89 Hz), 123.08, 124.22, 126.99, 133.43, 134.35, 137.28 (aromatic), 168.65 ($\underline{C}$=O), 195.52 ($\underline{C}$=S); m/z 285 (M^+), 189, 176 (base).

2-(Hydroxymethyl)-1[H]-Isoindole-1,3,(2H)-dithione

Dithiophthalimide (179mg, 1mmol) was heated at reflux in 40% aqueous formaldehyde (7.5ml) and water (25 ml). The mixture was refluxed for 8 hrs, cooled to room temperature and extracted with ethyl acetate (2 x 20 ml). The organic layer was dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a brown solid. Chromatography on silica eluting with dichloromethane yielded a red crystalline solid (153 mg, 74%) m.p. 118-120°C (from toluene). (Found: C, 51.46; H, 3.48; N, 6.69. $C_9H_7NOS_2$ requires C, 51.7; H, 3.4; N, 6.7%);

ν_{max} ($CHCl_3$) 3580(OH), 1600 (aromatic), 1350 (C=S)cm^{-1};

λ_{max} (EtOH) 377nm (ε 33,800), 240nm (ε 22,154);

δ_H (60MHz; $CDCl_3$) 3.3 (1H, br s, -OH exch. D_2O), 5.7 (2H, s, CH_2) 7.4-7.9 (4H, m, aromatic); δ_C(CD_3COCD_3) 66.41 ($\underline{C}H_2$), 124.11, 134.78, 135.67 (aromatic), 197.65 ($\underline{C}$=S);

m/z 209 (M^+), 179, 146 (base).

2-(Bromomethyl)-[H]-Isoindole-1,3,2(H)-dithione

2-(Hydroxymethyl)-1-[H]-Isoindole-1,3-(2H)-dithione (80 mg, 0.38 mmol) in dry dichloromethane (15 ml) at room temperature was stirred with phosphorus tribromide (36μ l, 0.38 mmol) for 3 hrs. The organic solution was washed twice with water (2 x 5 ml) dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a black solid. Chromatography on silica eluting with dichloromethane yielded a black crystalline solid (47 mg, 45%) m.p. 109-111°C (from toluene). (Found: C, 40.09; H, 2.25; N, 5.29; S, 23.13; Br, 29.30. $C_9H_6NS_2Br$ requires C, 39.85; H, 2.21; N, 5.16; S, 23.66; Br, 29.48%); $\nu_{max}(CHCl_3)$ 1600 (aromatic), 1350 (C=S) cm^{-1}; λ_{max} (EtOH) 355nm (ε 18,700), 250nm (ε20,400); δ_H(60MHz:$CDCl_3$) 6.05 (2H, s, CH_2Br), 7.4-7.8 (4H, m, aromatic); δ_C ($CDCl_3$) 36.95 ($\underline{C}H_2Br$), 123.57, 133.81, 135.33 (aromatic), 194.22 ($\underline{C}$=S); m/z 278(M^+), 271(M^+), 192 (base), 146.

1-(1,3-Dihydro-1,3-dithio-2H-isoindol-2-yl)methyl dimethyl phosphonate (163)

2-(Bromomethyl)-1- [H]-Isoindol-1,3,2(H)-dithione (136 mg, 0.5 mmol) in dry xylene (20 ml) was heated at reflux with trimethylphosphite (60 µl, 1.1 eq) for 8 hrs. The solvent was removed in vacuo and the crude mixture chromatographed on silica eluting firstly with dichloromethane to remove any unreacted starting material. The product was eluted with dichloromethane/ ethyl acetate (75:25). The solvents were removed to yield a dark red solid (70 mg; 47%) m.p. 106-107°C (from toluene). (Found: M^+, 300.9968. $C_{11}H_{12}NO_3PS_2$ requires M, 300.9996); ν_{max} ($CHCl_3$) 3,000 (CH), 1600 (aromatic), 1350 (C=S), 1200 (P-OR), 1050 (P=O) cm^{-1}; λ_{max} (EtOH) 355nm (ε 19,095), 245nm (ε 17,085); δ_H(100MHz ; $CDCl_3$) 3.72 (6H, d, J_{PH} 11Hz, $P(OCH_3)_2$), 5.94 (2H, d, J_{PH} -2Hz, CH_2P), 7.5-7.9 (4H, m, aromatics); δ_C ($CDCl_3$) 28.39 and 35.70 ($\underline{C}H_2P$. J_{CP} 146.48Hz), 52.87 and 53.15 (P-O$\underline{C}H_3$, J_{CP} 6.94Hz), 123.4, 133.32, 134.68 (aromatic), 195.95 ($\underline{C}$=S); m/z 301(M^+), 268, 205 (base), 192, 164, 146, 120, 93.

Thionation of N-Carbethoxyphthalimide; Formation of N-Carbethoxymonothiophthalimide (165) and N-Carbethoxydithiophthalimide (166)

To a stirred suspension of N-carbethoxyphthalimide (2.19 g, 0.01 mol) in dry toluene Lawessons reagent (4.4 g, 1.1 eq) was added. The stirred mixture was heated at reflux for 8 hrs. The mixture was cooled to room temperature and any insoluble material removed by filtration. T.l.c. showed the presence of two coloured products, unreacted starting material and the by-product from Lawessons reagent after thionation. Chromatography on silica eluting with toluene yielded N-carbethoxymonothiophthalimide (165) as a pink solid (225 mg, 30% corrected). Further elution with toluene/petroleum ether (66:33) yielded N-carbethoxydithiophthalimide (166) as a black solid 133 mg, 16% corrected).

N-Carbethoxymonothiophthalimide (165) (Found: M^+ 235.0303. $C_{11}H_9NO_3S$ requires 235.0317); ν_{max} ($CHCl_3$) 1760 (CO_2Et), 1710 (CON-), 1600 (aromatic), 1320 (C=S) cm^{-1}; λ_{max} (EtOH) 327nm (ε 11,058), 288 nm (ε 15,686), 231 nm (ε 31,568); δ_H (60MHz:$CDCl_3$) 1.35 (3H, t, CH_3), 4.35 (2H, q, OCH_2), 7.60 (4H, m, aromatic); δ_C ($CDCl_3$)14.03 ($\underline{C}H_3$), 64.90 ($\underline{C}H_2$), 123.63, 124.55, 126.28, 134.14, 135.06 136.90 (aromatic), 149.30 ($\underline{C}OOEt$), 165.99 (-$\underline{C}$ON-), 192.75 ($\underline{C}$=S); m/z 235(M^+), 191, 163, 130, 103 (base.)

N-Carbethoxydithiophthalimide (166) (Found: M^+ 251.0075. $C_{11}H_9NO_2S_2$ requires M, 251.0056); ν_{max} ($CHCl_3$) 1740 (C=O), 1580 (aromatic, 1370 (C=S), 1320 (-OEt) cm^{-1}; λ_{max} (EtOH)

354nm(ε 4,471), 242nm (ε 3,294); δ_H (60MHz:$CDCl_3$) 1.40 (3H, t, CH_3), 4.47 (2H, q, CH_2), 7.70 (4H, m, aromatic); δ_C($CDCl_3$) 13.92 ($\underline{C}H_3$), 65.93 ($\underline{C}H_2$), 123.46, 133.81, 134.46 (aromatic), 150.25 ($\underline{C}$=O), 193.67 ($\underline{C}$=S); m/z 251 (M^+), 146 (base).

N-Dithiophthalimido phenylalanine methyl ester (166).

N-Carbethoxydithiophthalimide (85 mg, 0.34 mmol) in dry dichloromethane (15 ml) was stirred at room temperature with phenylalanine methyl ester hydrochloride (1 eq) and triethylamine. The solvents were removed in vacuo to yield a black solid. Chromatography on silica eluting with dichloromethane/petroleum ether (50:50) yielded two products (166) (30 mg, 31% corrected) and the product due to initial attack by phenylalanine methyl ester without displacement of the amino carbethoxy ester (30 mg).

For (166) ν_{max} ($CHCl_3$) 2900 (CH), 1750 (CO_2Me), 1350 (C=S) cm^{-1}; λ_{max} (EtOH) 327nm (ε 1,532), 290nm (ε 1,937), 231nm (ε 4,995); δ_H (60 MHz:$CDCl_3$) 3.65 (2H, d, CH_2Ph), 3.74 (3H, s, OCH_3), 5.75 (1H, t, CH), 7.20 (5H, m, aromatic), 7.65 (4H, m, aromatic).

For by-product ν_{max} ($CHCl_3$) 2900 (CH), 1790, 1740, 1690 (C=O), 1600 (aromatic), 1450 (OCH_3), 1370 (C=S); λ_{max} (EtOH) 341nm (ε 20,430), 312nm (ε 26,344), 233nm (ε 39,785); δ_H (60MHz:$CDCl_3$) 1.37 (3H, t, CH_3), 3.60 (2H, d, CH_2Ph), 3.72 (3H, s, OCH_3), 4.30 (2H, q, CH_2), 5.91 (1H, t, CH), 7.15 (5H, m, aromatic), 7.60 (4H, m, aromatic); δ_C($CDCl_3$) 14.19 (CH_3), 34.83 ($\underline{C}H_2Ph$), 52.5 ($O\underline{C}H_3$), 56.29 (CH), 63.06 (OCH_2), 123.52, 124.87, 125.58, 126.61, 128.17 129.31, 133.22, 136.57 (aromatics), 169.24 (C=O), 194.54 (C=S).

N-Monothiophthalimido phenylalanine (167)

N-Carbethoxymonothiophthalimide (225 mg, 0.95 mmol) in water (25 ml) was stirred at room temperature with phenylalanine (175 mg, 0.96 mmol) and sodium carbonate (115 mg, 0.96 mmol). The suspension was stirred for 5 hrs. The solution was extracted with dichloromethane (2 x 20 ml) and from the organic layer unreacted starting material (151 mg) was recovered. The solvent from the aqueous layer was removed in vacuo to yield a pink gum. Preparative thin layer chromatography on silica eluting with chloroform/methanol/acetic acid (90:10:1) yielded a pink solid (167) (50 mg, 51% corrected).

(Found: M^+ 311.0618 $C_{17}H_{13}NO_3S$ requires M, 311.0616); ν_{max} ($CHCl_3$) 3300-2700 (CO_2H), 1750, 1710 (C=O), 1600 (aromatic) 1350 (C=S) cm^{-1}; δ_H (60MHz:$CDCl_3$) 3.45 (2H, m, CH_2Ph), 5.76 (1H, m, CH), 7.08 (5H m aromatic), 7.60 (4H, m, aromatic); δ_C ($CDCl_3$) 34.62 ($\underline{C}H_2Ph$), 55.76 ($\underline{C}H$), 122.92, 124.11, 126.66, 128.29, 129.04, 133.22, 134.03, 136.57 136.74 (aromatic), 169.13 (C=O), 196.12 (C=S); m/z 311 (M^+), 168, 148, 84 (base).

N-Monothiophthalimido phenylalanine methyl ester (168)

N-Carbethoxymonothiophthalimide (151 mg; 0.64 mmol) in dry dichloromethane was stirred at room temperature with phenylalanine methyl ester hydrochloride (1 eq) and triethylamine (1 eq) for 5 hrs. The reaction mixture was extracted with water (2 x 20 ml) and 5% citric acid (2 x 20 ml). The organic layer was dried over magnesium sulphate, filtered and the solvent removed in vacuo to yield a red oil (120 mg). Chromatography on silica eluting with dichloromethane/petroleum ether (50:50) yielded (168) (60 mg, 29%) (Found: M^+ 325.0772 $C_{18}H_{15}ON_3S$ requires M, 325.0734); ν_{max} ($CHCl_3$) 2900 (CH), 1710 (C=O), 1580 (aromatic), 1420 (OCH_3), 1320 (C=S) cm^{-1}; λ_{max} (EtOH) 322nm (ε 1,200), 290nm (ε 1,626), 233nm (ε 4,146); δ_H (60MHz:$CDCl_3$) 3.60 (2H, d, CH_2Ph), 3.73 (3H, s, OCH_3), 5.70 (1H, t, $\underline{CH}$), 7.14 (5H, m, aromatic), 7.67 (4H, m, aromatic); δ_C ($CDCl_3$) 34.83 ($\underline{CH_2}Ph$), 52.77 ($O\underline{CH_3}$), 55.67 ($\underline{CH}$), 122.98, 124.22, 126.82, 128.45, 129.15, 129.86, 133.38, 134.24, 136.63, 136.95 (aromatic), 169.13 ($\underline{C}$=O), 196.33 ($\underline{C}$=S); m/z 325 (M^+), 162 (base), 131, 103.

Dimethyl isovaleryl phosphonate (169)

Isovaleryl chloride (12.2 mol, 0.1 mol) was added dropwise to trimethyl phosphite (12.5 ml, 0.11 mol) with stirring under an argon atmosphere at room temperature. The reaction mixture was stirred at room temperature overnight. Distillation of the reaction mixture afforded dimethyl isovaleryl phosphonate (13.3 g, 69%). B.p. 108 - 111°C 5mm Hg; ν_{max} (neat) 2950 (CH), 1700 (C=O), 1270 (P-OCH_3), 1050 (P=O) cm^{-1}, δ_H (60MHz: $CDCl_3$) 0.92 (6H, d, $(CH_3)_2$), 2.20 (1H, m, CH-), 2.70 (2H, d, CH_2), 3.85 (6H, d, J_{PH} 12Hz, P-OCH_3); m/z 195 (M+1), 194 (M^+), 109 57 (base).

Isovaleryl phosphonic acid (173)

Dimethyl isovaleryl phosphonate (1 g, 5 mmol) in dry dichloromethane under an argon atmosphere was treated with bromotrimethylsilane (2.01 ml, 15 mmol) at room temperature. The mixture was stirred for 3 hrs and the solvent removed in vacuo to yield a yellow oil. This oil was treated with acetone/water (9:1) (10 ml) and the mixture stirred at room temperature overnight. Solvents were removed under reduced pressure to yield a colourless hygroscopic oil (0.6 g, 70%) ν_{max} ($CHCl_3$) 3300-2700 (P-OH), 1710 (C=O), 1200 (P-OCH_3), 1030 (P=O) cm^{-1}; δ_H (60 MHz:D_2O) 0.94 (6H, d, $(CH_3)_2$), 2.20 (1H, m, CH-), 3.78 (2H, d, CH_2CO); δ_C (D_2O) 22.32 ($(\underline{C}H_3)_2$), 23.89 and 23.99 ($\underline{C}H(CH_3)_2$) J_{CP} 2.44 Hz), 50.71 and 52.82 ($\underline{C}H_2COP$, J_{CP} 47.6Hz), 178.18 (C=O); m/z 166(M^+), 119 (base).

α-Hydroxy- γ-methylbutyl phosphonic acid (174).

Dimethyl isovaleryl phosphonate (1.093 g, 5.6 mmol) in dichloromethane (20 ml) was treated with sodium borohydride (0.56 g). The mixture was stirred at room temperature for 2 hrs. The stirred solution was diluted with water (20 ml) and stirred for a further 1 hr. The two layers were separated and the aqueous layer washed twice with dichloromethane (2 x 30 ml). The combined organic extracts were dried over magnesium sulphate, filtered and the solvent removed in vacuo to yield a colourless oil (0.79 g, 72%).

ν_{max} (neat) 3600-3200 (OH), 1220 (P-OCH_3), 1050 (P=O) cm^{-1}; m/z 195 (M-1), 110 (base).

The dimethyl α-hydroxy-γ methylbutyl phosphonate (0.5 g, 2.55 mmol) in dry dichloromethane under an argon atmosphere was treated with bromotrimethylsilane (1.02 ml, 7.65 mmol) at room temperature. The mixture was stirred for 3 hrs and the solvent removed in vacuo to yield a colourless oil. This oil was treated with acetone/water (9:1) (10 ml) and the mixture stirred at room temperature overnight. Solvents were removed under reduced pressure to yield a white solid (0.37 g, 85%) m.p. 215-217°C; Found: C, 33.89; H, 7.97. $C_5H_{12}O_4P.\frac{1}{2}H_2O$ requires C, 33.80; H, 7.59%; δ_H (100MHz; $(CD_3)_2SO$) 0.85 (6H, m, $(CH_3)_2$), 1.2-1.9 (4H, complex, $\underline{CH}\underline{CH_2}$ and -$\underline{OH}$), 3.60 (1H, m, $\underline{CH}OH$), 8.58 (1H, br, $PO_3\underline{H_2}$); δ_C ($(CD_3)_2SO$) 21.30 ($(CH_3)_2$), 24.79 ($\underline{CH}CH_2$), 38.52 and 40.42 ($\underline{CH_2}CHOHP$, J_{CP} 40.42 Hz), 61.55 and 68.75 ($\underline{CH}OHP$, J_{CP} 162.35Hz); m/z [+] ion FAB 169 (M+1) (base), 191 (M+Na); [-] ion FAB 167 (M-1) base.

Dimethyl α-oximino- γ-methylbutyl phosphonate (171)

Dimethyl isovaleryl phosphonate (9.813 g, 0.051 mol) in ethanol (10 ml) was treated with hydroxylamine hydrochloride (4.6 g, 0.066 mol) and pyridine (5.6 g). The mixture was stirred at room temperature for 24 hrs. The solvent was removed in vacuo to yield a colourless oil to which 2M hydrochloric acid (20 ml) was added. The aqueous solution was extracted with dichloromethane (5 x 20 ml) and the combined organic extracts were washed with 2M hydrochloric acid (6 x 10 ml), water (2 x 10 ml), saturated sodium bicarbonate (2 x 10 ml) and water (2 x 10 ml). The organic solution was dried over magnesium sulphate, filtered and the solvent removed in vacuo to yield a colourless gum (6 g, 56%). ν_{max} (neat) 3400 (OH), 2950 (CH), 1470 (C=N), 1250 (P-OCH_3), 1050 (P=O) cm^{-1}; δ_H (60MHz:$CDCl_3$) 0.95 (6H, m, $(CH_3)_2$), 2.40 (3H, m, $CHCH_2$), 4.08 (6H, 2d, J_{PH} 11Hz, P-OCH_3); δ_C ($CDCl_3$), 22.31, 22.75 ($(\underline{C}H_3)_2$), 26.38, 26.65 ($\underline{C}HCH_2$), 34.73 and 41.17 ($\underline{C}H_2$, J_{CP} 145.3 Hz), 34.45 and 41.93 ($\underline{C}H_2$, J_{CP} 145.3Hz), 52.87 and 53.31 (P-$O\underline{C}H_3$, J_{CP} 9.77), 53.15 and 53.58 (P-$O\underline{C}H_3$, J_{CP} 9.77Hz), 146.49 and 153.21 ($\underline{C}$=N, J_{CP} 151.36Hz), 147.84 and 157.38 ($\underline{C}$=N, J_{CP} 214.85Hz); δ_P ($(CD_3)_2SO$), 8(M), 12(M), m/z 210 (M+1) (base), 190, 110, 84.

α -Oximino-γ -methylbutyl phosphonic acid (175)

Dimethyl α-oximino-γ -methylbutyl phosphonate (2.88 g, 13.8 mmol) in dry dichloromethane (20 ml) was treated with bromotrimethylsilane (1.84 ml, 41.4 mmol) and the mixture was stirred for 3 hrs. The solvent was removed in vacuo to yield a colourless gum which was dissolved in acetone/water (9:1) (10 ml) and the solution stirred at room temperature for 30 mins. Water (20 ml) was added to the

mixture and the aqueous solution extracted with dichloromethane (2 x 20 ml). The organic extracts were discarded and the water from the aqueous solution was removed in vacuo to yield a colourless hygroscopic gum (600 mg; 24%) δ_H (60MHz:D_2O) 0.92 (6H, d, $(CH_3)_2$), 2.11 (1H, m, $\underline{CH}CH_2$), 2.30 (2H, d, $CH\underline{CH_2}$); δ_C (D_2O) 21.32 ($(\underline{CH_3})_2$), 21.43 ($\underline{C}HCH_2$), 42.88 and 43.26 ($\underline{CH_2}CP$, J_{CP} 8.55Hz), 178.42 and 180.21 ($\underline{C}$=N); m/z (M+1) 182 [+] ion FAB, (M-1) 181 [-] ion FAB.

Dimethyl α-amino-γ methylbutyl phosphonate (172)

Dimethyl α-oximino-γ-methylbutyl phosphonate (4.97 g, 23.7 mmol) in ethanol (200 ml) containing Raney nickel (3 g) was hydrogenated at 75 atmospheres and 100°C for 10 mins. The mixture was allowed to cool to room temperature and filtered through Celite. The solvent was removed in vacuo to yield a pale yellow oil. Chromatography on silica eluting with chloroform/methanol (9:1) yielded a colourless gum (3.85 g, 85%). ν_{max} (neat) 3450 (NH_2), 2950 (CH), 1250 (P-OCH_3), 1050 (P=O) cm^{-1}, δ_H (100MHz:$CDCl_3$) 0.95 (6H, m, $(CH_3)_2$), 1.55 (3H, m, $CHCH_2$), 3.18 (1H, dt, $\underline{CH}P$, J_{PH} 11Hz), 3.80 (6H, d, P-OCH_3, J_{PH} 11Hz), 4.80 (2H, br s, NH_2); δ_C ($CDCl_3$) 21.24, 23.30 ($(CH_3)_2$), 21.67 ($\underline{CH}$ CH_2), 39.71 $CH\underline{CH_2}$), 42.74 and 49.41 ($\underline{CH}P$, J_{CP} 150.15Hz), 52.82 and 53.15 (P-$O\underline{CH_3}$ J_{CP} 7.32), 52.98 and 53.25 (P-$O\underline{CH_3}$, J_{CP} 6.11Hz).

α -Amino-γ -methylbutyl phosphonic acid

Dimethyl α-amino-γ-methylbutyl phosphonate (1 g, 5.1 mmol) in dry dichloromethane (20 ml) was treated with bromotrimethylsilane (0.11 ml, 20.5 mmol) and the mixture was stirred at room temperature for 3 hrs. The solvent was removed in vacuo to yield a colourless gum which was dissolved in acetone/water (9:1) (10 ml) and the solution stirred at room temperature for 30 mins. The solvents were removed in vacuo to yield a white solid which was recrystallised from methanol-water to yield a colourless crystalline solid (0.52 g, 61%) m.p. 278-281°C (Lit.[185] 279-280°C).

N-Benzyloxycarbonyl-L-phenylalanyl- α-amino-γ-methylbutyl phosphonate dimethyl ester (176).

N-Benzyloxycarbonyl-4-phenylalanine (3.7 g, 13 mmol) in dry dichloromethane (50 ml) at -23°C was treated with diphenyl phosphinyl chloride (DPPCl) (3.07 g, 13 mmol) and N-methylmorpholine (1.43 ml, 14.3 mmol). The mixture was stirred at -23°C for 30 mins. after which dimethyl α -amino-γ -methylbutyl phosphonate (2.54 g, 13 mmol) was added to the stirred mixture. The reaction mixture was allowed to warm to room temperature and stirred for 8 hrs. The mixture was then washed with saturated sodium bicarbonate (2 x 20 ml), 2M hydrochloric acid (2 x 20 ml) and water (2 x 20 ml). The organic layer was dried over magnesium sulphate, filtered and the solvent removed in vacuo to yield a pale yellow gum. Chromatography on silica eluting with ethyl acetate yielded a colourless gum (4.206 g, 70%). ν_{max} (neat), 3300 (NH), 2950 (CH), 1720, 1690 (C=O), 1600 (aromatic), 1250 (P-OCH_3), 1050 (P=O); δ_H (60MHz:$CDCl_3$) 0.85 (6H, m, $(CH_3)_2$), 1.45 (3H, m, $CHCH_2$), 3.12 (2H, m, $C\underline{H}_2Ph$), 3.78 (6H, d, J_{PH} 11Hz), 4.75 (3H, br, NH, 2 x NHC$\underline{H}$), 5.04 (2H, s, $C\underline{H}_2OPh$), 6.05 (1H, tr, N$\underline{H}$), 7.25 (10H, m, aromatic).

L-Phenylalanyl-α-amino-γ-methylbutyl phosphonic acid (177)

N-Benzyloxycarbonyl-L-phenylalanyl-α amino-γ-methylbutyl phosphonate dimethyl ester (2.33 g, 5 mmol) in dichloromethane (10 ml) was treated with hydrogen bromide in glacial acetic acid (10 ml). The mixture was stirred at room temperature for 30 mins. The solvent was removed in vacuo and the residue dissolved in ethyl acetate. The organic layer was washed with water and a gel formed in the aqueous layer. The aqueous solution was filtered and the gel was freeze dried to yield a white solid (1.23 g, 78%). m.p. 287-289°C; δ_H (100MHz:D_2O), 0.80 (6H, m, $(CH_3)_2$), 1.53 (3H, m, CH_2CH), 3.20 (2H, m, $\underline{CH}_2Ph$), 4.27 (1H, m, $\underline{CH}P$), 7.31 (5H, m, aromatic); δ_C ($(CD_3)_2SO$), 20.64 ($(CH_3)_2$), 21.02 ($\underline{CH}_2CH$), 23.24 ($\underline{CH}_2Ph$), 51.42 and 52.98 ($\underline{C}HP$, J_{CP} 110Hz), 127.56, 128.61, 129.31, 129.56, 134.46 (aromatic), 169.9 (C=O); m/z (M+1) 315 [+] ion FAB, (M-1) 313 [-] ion FAB.

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